Report of

The Medical Commission for the Investigation of Acute Respiratory Diseases of the Department of Health of the City of New York

Part I

Studies on the Pneumococcus

Reprinted from

The Journal of Experimental Medicine, 1905

Vol. VII, No. 5

The Knickerbocker Press
(G. P. Putnam's Sons)
New York

CONTENTS

PREFACE.

For some years the problem presented by the very large and constantly increasing death-rate from the acute respiratory diseases has been the cause of serious concern to the Board of Health of New York City, and in August, 1904, with the consent of the Mayor and the Board of Estimate, it appointed a Medical Commission to conduct an investigation as to the causes for the great prevalence of the acute respiratory diseases in the city, with the hope that some means could be devised for reducing the excessive morbidity and mortality from this cause.

The problem has long been recognized as one of the first in sanitary importance, and efforts have frequently been made to find some method for its solution. The inherent difficulties of the problem, however, are so great and the other questions pressing for consideration have been so numerous and important that no determined efforts have hitherto been made to discover means or devise methods to effectually meet the situation.

Succinctly stated, the situation is this: During the last twenty years the general death-rate in New York City has fallen 25 % and the death-rates from all the principal causes of deaths have fallen from 10 % to 35 %, excepting in four groups of diseases, in which there has been an increase. These are, *first*, the acute respiratory diseases; *second*, cancer; *third*, the diseases of the heart and blood-vessels; *fourth*, the diseases of the kidney. The increases in these groups have been from 10 % to 30 % or 35 %,—in the acute respiratory being from 10 % to 15 %. In the first half of 1904 there was an extraordinary increase in this group, the deaths from the acute respiratory diseases alone forming no less than 23 % of the total death-rate.

The importance of the situation, so far as this group is concerned, is more forcibly brought out when we compare the percentage which the total deaths from the acute respiratory diseases bear to the total deaths from all causes. In 1883, it

was between 7 and 8 %, and in 1893 nearly 16 %, showing a relative increase during this period of 100 %.

A consideration of the etiology of the acute respiratory diseases brings out even more strongly the sanitary importance of the problem. There can now be no question that the exciting cause in each one of the diseases of this group is a micro-organism, which requires such conditions for its growth and multiplication as are usually found only in the living body. These organisms do not under natural conditions multiply to any extent outside of the tissues or cavities of the body. The infection, when it occurs, must practically always be the result of communication, directly or indirectly, from one human being to another. The conclusion, therefore, seems justifiable that these diseases are essentially communicable, and however great the inherent difficulties of the problem may be, theoretically at least, they should be to a greater or less extent preventable.

The difficulties in the way of prevention arise largely from the wide distribution of the organisms. Streptococci, pneumococci, influenza bacilli, singly or combined, are present in almost all morbid conditions in the respiratory tracts of the inhabitants of large cities, and probably, also, to a very large extent in the respiratory tracts of healthy individuals. The situation is much like that existing with relation to diphtheria, excepting that these organisms probably live more readily and for longer periods of time in the body cavities of healthy individuals than do the diphtheria bacilli, and the latter perhaps have (at least in children) greater pathogenic activity.

The Board of Health, looking forward to the study of this problem in the revision of the Sanitary Code made in 1903, included this group of diseases in the class in which partly voluntary and partly compulsory notification was required. This provision of the Sanitary Code, however, has not yet been enforced. It is believed now that provision should be made for this.

It has always been the feeling of the Medical Officers of the Board, that no really important effective measure could be taken in relation to any preventable disease unless the Sanitary

authorities first had some fairly comprehensive information as to the prevalence and distribution of the disease—such information as can only be gathered from the systematic notification of cases.

As the problem under consideration is not simply one which concerns New York, but one which almost equally concerns all the large cities of the United States, it was felt to be important that the Commission should have a distinctly representative character, in the hope that the influence which it would exercise would be broader and more effective. As it seemed desirable to the Commission to carry on investigations in other laboratories in other cities, suitable arrangements were made for defraying the expenses of these investigations by the Department of Health of New York City. The facilities of the laboratories of the Department of Health were also placed at the command of the Commission for assisting in various lines of investigation in New York.

The Commission was constituted as follows:

Dr. Edward G. Janeway, of New York, *President.*
Dr. William Osler, of Baltimore, *Vice-President.*
Dr. T. Mitchell Prudden, of New York, *Secretary.*
Dr. Theobald Smith, of Boston.
Dr. William H. Welch, of Baltimore.
Dr. Frank Billings, of Chicago.
Dr. John H. Musser, of Philadelphia.
Dr. L. Emmett Holt, of New York.
Dr. Francis P. Kinnicutt, of New York.

(Signed)

Thomas Darlington, M.D.,
President.

Dr. Hermann M. Biggs,
General Medical Officer,
Department of Health,
New York City.

INTRODUCTORY NOTE TO STUDIES ON THE PNEUMOCOCCUS UNDER THE AUSPICES OF THE MEDICAL COMMISSION FOR THE INVESTIGATION OF ACUTE RESPIRATORY DISEASES OF THE DEPARTMENT OF HEALTH OF THE CITY OF NEW YORK.

The Medical Commission for the Investigation of Acute Respiratory Diseases, which was formed at the request of the Department of Health of the City of New York, began its work in October, 1904.

It was decided to concentrate attention at first upon lobar pneumonia in both its bacteriological and its clinical aspects. Among the studies upon the bacterial excitant of lobar pneumonia which it was deemed wise to pursue were the following:

1. A study of the occurrence and virulence of pneumococcus and organisms related to or resembling this, in the human mouth in health and disease.

2. The evidence of variations in virulence of the pneumococcus.

3. The occurrence of the pneumococcus in children's hospitals, homes, and asylums, with a study of the bacteria of mouths before and after outbreaks of pneumonia.

4. The vitality of the pneumococcus under various conditions.

5. A study of mouth disinfection.

It was the sense of the Commission that one of the earliest phases of research, upon which much of the subsequent work would depend, was the determination, for the identification of species, of the characters of the pneumococcus, this investigation to include a comparative study of the coccus from cases of true lobar pneumonia and of that from normal mouths and throats hitherto generally assumed to be identical with the pneumococcus, also to include such a study of streptococci as will suffice for their separation from the pneumococcus.

The method adopted in these studies was, first, to secure the coöperation of bacteriologists in various places, who should make independent studies along the lines suggested; and, second, to establish a central laboratory or "clearing house" to which ultimately cultures from various independent workers should be sent for comparative study under a single observer. It was hoped that by the establishment of this central laboratory with its large number of available cultures, light might be thrown upon atypical forms, variations, etc., and greater precision achieved in determining the characters relied upon for identification.

The Commission secured the coöperation, in making the special studies referred to, of Dr. W. T. Longcope, of the Ayer Clinical Laboratory in Philadelphia, of Dr. C. W. Duval and Dr. Paul Lewis of the City Hospital in Boston, of Dr. William H. Park and his associates in the laboratories of the Department of Health of New York City, of Dr. Leo Buerger, of Mt. Sinai Hospital, New York, of Prof. F. C. Wood, of the College of Physicians and Surgeons, New York, and Dr. Charles Norris, of New York.

The coöperation of Prof. Philip Hanson Hiss, of the College of Physicians and Surgeons, New York, was secured in the organization and direction of the work of the central laboratory at the College of Physicians and Surgeons.

While various other lines of study are under way, it has been thought wise to publish at this time together the several independent studies which follow, in the belief that they may be useful not only in the further work of the Commission but of others engaged in similar lines of research.

A STUDY OF PNEUMOCOCCI: A COMPARISON BETWEEN THE PNEUMOCOCCI FOUND IN THE THROAT SECRETIONS OF HEALTHY PERSONS LIVING IN BOTH CITY AND COUNTRY AND THOSE OBTAINED FROM PNEUMONIC EXUDATES AND DISEASED MUCOUS MEMBRANES.

By WILLIAM H. PARK, M.D., Director,
and
A. W. WILLIAMS, M.D., Assistant Director,

assisted by

A. OPPENHEIMER, C. BOLDUAN, M.D., J. L. BERRY, M.D., M. A. ASSERSEN, M.D., M. LOWDEN, M.D., and I. VAN GIESEN, M.D.

(*From the Research Laboratory of the Health Department of New York City.*)

The investigations carried on in the Research Laboratory were planned after consultation with the members of the Commission for the Investigation of Acute Respiratory Diseases, of the Health Department of the City of New York, but were otherwise entirely independent of that body. The study of the agglutination characteristics was undertaken by Dr. K. R. Collins, whose report follows this. The investigations are still being carried on and these preliminary reports are made at the suggestion of the Commission, so that all the workers in carrying on further studies might receive help from work already done.

PLAN OF INVESTIGATIONS.

In this study the following points have been considered:

I. The presence of pneumococci (1) in normal sputum, (2) in pneumonic sputum and autopsy material, (3) in the sputum or exudates from pathogenic cases other than pneumonias.

II. The comparison of the strains obtained from the different sources in the following particulars: (1). Morphological and cultural characteristics. (2). Virulence. (3). Serum reactions.

The scheme of the work, which was carried out more or less fully, is tabulated as follows:

TABLE I.

Original material from autopsy, sputum or other source.

Three smears made:

1. Stained with Loeffler's methylene blue.
2. Stained with Gram's solutions.
3. Stained with Hiss' capsule stain.

Remove a certain portion aseptically and plant into serum-broth (A). From tube A make several dilutions, tubes B, C, D, etc.

After 24 hrs. at 36° C. make smears from each tube, select culture most characteristic of pneumococcus, and inject subcutaneously into

Rabbit (800–1000 gms.)
3 or 4 c.c.

or

White mouse (young adult)
½ c.c.

When animal dies, expose heart and inoculate heart's blood into serum-broth, over surface of agar plate and into Hiss' inulin medium. Also make three smears stained as in beginning.

After 24 hrs., if the serum-broth shows pure culture of pneumococcus or of streptococcus-like organisms, inoculate into two rabbits if from rabbit or into two mice if from mouse, to test for virulence. If culture is not pure, fish from the pneumococcus-like colonies on agar plate. When pure culture is thus obtained, inoculate as above.

(Keep this culture for stock strain.)

Rabbit (800–1000 gms.)
4 c.c. into ear vein

and

Rabbit (800–1000 gms.)
$\frac{1}{10}$ c.c. into ear vein

or

White mouse (young adult)
$\frac{1}{100}$ c.c. subcutaneously

and

White mouse (young adult)
$\frac{1}{1000}$ c.c. subcutaneously.

At autopsy make from heart's blood a culture into serum-broth, over a surface agar plate and into Hiss' inulin medium. Make three smears, stained as in the beginning.

STOCK STRAINS. — Plant these into various culture media and study their characteristics. Replant the stock cultures on slant blood-agar every four to seven days, keeping track of the number of culture generations. Study minutely from time to time in the various media, in animals and in serum reactions. Note any changes.

Make four blood-agar plates, one direct from original material, and one each from dilution tubes A, B, and C.

After 24 hrs. at 36° C. describe gross appearance of plates and colonies. Study under 2 and 7 magnification and note proportion of pneumococcus-like colonies to others. Fish from 10 to 15 pneumococcus-like colonies and plant on slant blood-agar. (If the original material was absolutely fresh, the colonies in the majority, whether pneumococcus-like or not, should also be fished.) If streptococcus-like colonies are present on plate, fish a few and carry on with the others.

After 24 hrs. transfer from slant blood-agar to serum-broth.

Make smear and note morphology in serum-broth from each of these fishings. Inoculate 0.5 c.c. of each into a tube of Hiss' inulin medium. Observe proportion of cultures which coagulate the inulin medium, and time required. Save distinct varieties of pneumococci and certain strains of streptococci for permanent strains.

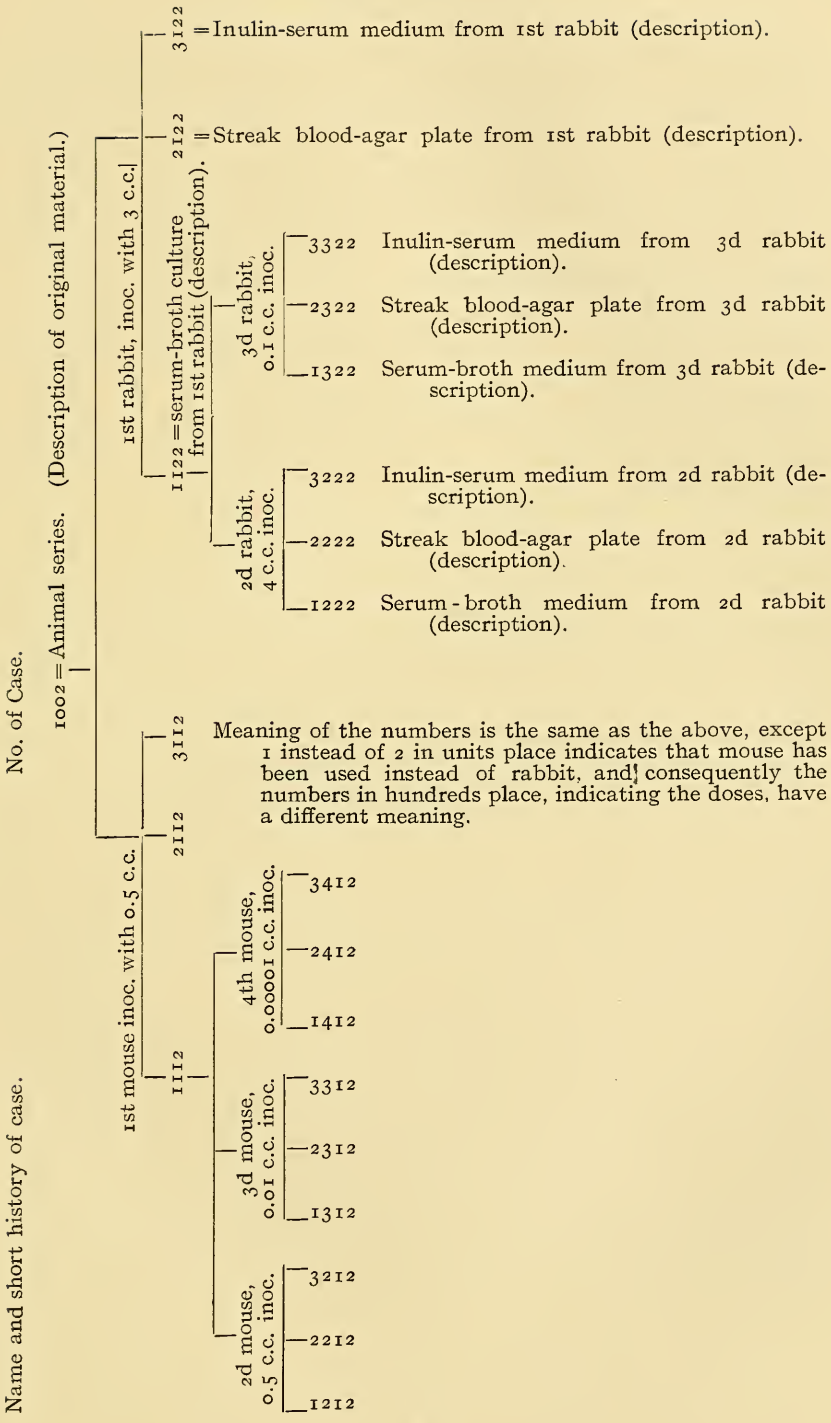
(Keep this culture for stock strain.)

Test the virulence of this culture or cultures, both in rabbits and white mice, following the scheme given above.

At autopsy make cultures and smears as indicated above.

TABLE II.

GENEALOGICAL RECORD.



Meaning of the numbers is the same as the above, except 1 instead of 2 in units place indicates that mouse has been used instead of rabbit, and consequently the numbers in hundreds place, indicating the doses, have a different meaning.

Keeping of Records.—Genealogical tables of each strain have been kept, a modification of the Dewey Library System of numbers being used to indicate the cultures. Thus by referring to these tables one is able quickly to get the principal points in the history of a particular culture from the time of its isolation.

Numbers of four denominations have been used, the units place indicating the series, the tens place the animal used for inoculations, the hundreds place the dose received, and the thousands place the medium employed. Underneath this number the number of culture generations is placed in parenthesis. Table II is one such genealogical table.

In addition to these tables, comment sheets on each strain and tables of comparative morphology and cultural peculiarities have been kept.

THE PRESENCE OF PNEUMOCOCCI.

Two hundred cases have been examined for the presence of the pneumococcus. In the great majority of cases two methods—(*a*) animal inoculation of mass cultures and (*b*) stroke blood-agar plates, as shown in Table I—have been employed in attempts at isolation; in the other cases only one of these two methods has been used. Table III shows the grouping of the cases and the number in which typical and atypical pneumococci have been found.

From this table we see that typical pneumococci have been obtained in a large percentage of normal cases in both city and country. A few pneumococci may have been missed because of occasional contaminations or overgrown cultures or the employment of large rabbits or some other cause. In the majority of cases where no pneumococci were found streptococci were isolated. From a series of autopsies on cases of broncho-pneumonia at the Willard Parker Hospital, and from a series of pertussis sputa from the Foundling Hospital, large numbers of influenza-like organisms were found with smaller numbers of streptococci and occasionally with a few pneumococci. It was very difficult to get rid of these influenza-like organisms, as great numbers passed through the animal inoculated with mass-cultures, and in the plates and serum-broth tubes they grew

abundantly in close association with the pneumococcus. Repeated platings generally had to be made before a pure culture of the pneumococcus could be obtained in these cases.

TABLE III.

SHOWING NUMBER OF CASES STUDIED AND NUMBER IN WHICH PNEUMOCOCCI WERE FOUND.

Groups.	Subdivisions.	Number of Cases.	Pneumococci not Isolated.	Pneumococci Isolated.	
				Atypical.	Typical.
Normal.	Research Laboratory...	3	3		
	Bellevue Students	10	2	1	7
	Saranac Lake	28	15	3	11
	Sea Breeze.....	5	3		2
	Tarrytown.....	1			1
	Foundling Hospital ...	9	2		7
	Briarcliff.....	6	3		3
	Hyde Park	4		4	
	Millbrook.....	7		2	5
	Newburgh	5		1	4
	Babies' Hospital.....	2	2		
Pneumonia.	Lobar-..	53	4	5	45
	Broncho-	21	5	2	14
Colds.		15	5		10
Miscellaneous.	Measles.....	3	1		2
	Scarlet-fever	5	3		2
	Tuberculosis	5	3		2
	Pertussis.....	5	4		1
	Influenza	2	2		
	Pleurisy	1			1
	Typhoid.....	1	1		
	Mastoiditis.....	1			1
	Synovitis	3	1		2
	Meningitis	1			1
	Œdema of Lungs.....	1	1		
	Empyæma	3	1		2

COMPARISON OF STRAINS.

Morphological and Cultural Characteristics.—We have divided the pure cultures of pneumococci obtained into two broad groups according to their morphological and cultural characteristics. The first group is composed of typical pneumococci and the second of atypical ones.

By typical pneumococci we mean cocci which (1) under certain more or less constant cultural conditions occur principally in slightly elongated and pointed twos with broader ends

apposed, (2) under similar or other cultural conditions form capsules, (3) when grown in inulin-serum [1] medium produce coagulation, and (4) when grown in poured blood-agar plates produce a distinct green color in and about colonies.

By atypical pneumococci we mean (1) cocci which morphologically and culturally resemble more or less closely the pneumococcus except in their growth in the Hiss inulin medium, which they do not coagulate; (2) cocci which are like streptococci morphologically, but which produce coagulation of the Hiss inulin medium.

Referring to Table III we see that a larger number of atypical strains have been obtained from normal cases, in all of which only sputum was studied, than from pathogenic. This may be due simply to the fact that so many more typical pneumococci were present in the majority of pathogenic cases studied that the atypical ones may have been missed in some of these cases. Atypical pneumococci of the first group, i.e., those which do not coagulate inulin-serum medium, have been found as the majority of colonies and as the only pneumococcus-like organism in the sputum from two cases of pneumonia, and have been accompanied by typical pneumococci in the sputum from three other

[1] The inulin used in the course of the present work in making up Hiss' medium (*Jour. of Exper. Med.*, 1905, vii, 317) was prepared in this laboratory by R. B. Gibson, for at the time we were unable to obtain it from commercial sources. The method employed in the obtaining of this substance follows: Dandelion (taraxacum) roots were soaked in cold tap water until soft, and if coarsely ground the roots were then run through an ordinary hash machine. The material was transferred to a gauze bag, and was washed thoroughly in running cold tap water to remove a portion of the soluble impurities and the finer solid particles which would interfere with subsequent filtration. The washed roots were extracted in boiling water, strained, and filtered. A second immediate extraction followed. The filtrates were united and evaporated over a Bunsen burner to a thin syrup. Alcohol (10–15 %) was added and the mixture was cooled to 0° or below. The inulin separating out on standing was thoroughly washed by decantation with cold alcohol (10–20 %) and then with 95 % alcohol. It was filtered into a suction funnel, washed on the filter with hot alcohol, sucked dry, and finally spread on filter paper in a warm place to remove the alcohol still remaining. The resulting product can be obtained as a fine white powder which gives the ordinary reactions of inulin; solutions of this product do not reduce Fehling's solution. The yield from five pounds of the tubers was about three hundred grams.

cases. So far they have not been found in autopsies following pneumonia. In one autopsy case and in one broncho-pneumonic sputum large numbers of cocci of the second group of atypical pneumococci were found.

It is interesting to note that, when some of the non-coagulating cultures were studied more minutely, various colonies being fished and the resulting cultures being tested for their ability to coagulate serum-inulin media, in the case of one culture one colony out of six produced late coagulation. From this coagulating colony, however, no further colonies were obtained producing coagulation. Among the typical pneumococci all strains vary somewhat with regard to their power to coagulate the Hiss medium, a few producing very late coagulation. When individual colonies were fished from some of these latter strains, it was found that there was a wide variation in the time required for coagulation, an occasional one not coagulating at all. It seems, from these observations, that the non-coagulating, more or less morphologically typical pneumococci are closely related to the typical late coagulators. One of these atypical strains showed typical capsules in the heart's blood of animals and the others showed occasional small capsules.

All of the typical and atypical strains, as well as many strains of streptococci, have produced a green color in and about colonies in poured blood-agar plates, while other streptococci have produced large areas of hæmolysis about colonies and no green color. These results agree in part with those of Schottmüller (*Münchener med. Woch.*, 1903, p. 849), and E. Fränkel (*Münchener med. Woch.*, 1905, p. 548), who divide streptococci into three groups according to their behavior in blood-agar plates: Streptococcus pyogenes producing much hæmolysis, Streptococcus virideus producing green color, and Streptococcus mucosus producing mucous-like material as well as green color. These results differ from those of Rosenow (*Journal of Infectious Diseases*, 1904, I, 280) who states that no streptococcus tried by him produced green color, while all pneumococci did, and he therefore recommends this test in differentiating the two species. From the sputa of a number of cases of broncho-

pneumonia we tried to isolate the pneumococcus by this method, making poured blood-agar plates from different dilutions of the sputum and fishing from the green colonies, and at the same time we used the method of animal inoculation by mass-cultures. In every case by the first method only streptococcus-like organisms were obtained, while by the second typical pneumococci were isolated.

All of the strains of typical pneumococci studied by us may be divided into several distinct morphologic varieties. We call them varieties, because while each strain shows a wide limit of fluctuating variability, certain strains have similar predominating constant characteristics. These varieties are:

1. Small cocci occurring under most cultural conditions in twos and producing small capsules.

2. Large cocci occurring readily in short and medium-length chains and producing large capsules.

3. The so-called Streptococcus mucosus.

The first two varieties are less distinctly bounded than the last which forms a definite morphologic variety. This variety, which has been mentioned only a few times in literature (Schottmüller, *Münchener med. Woch.*, 1903; L. Buerger, *Medical News*, 1904, p. 1117; E. Fränkel, *Münchener med. Woch.*, No. 12, 1905; L. Heim, *Zeit. für Hyg.*, 1905, I, 139), has been classed as a streptococcus, under the name of Streptococcus mucosus by Schottmüller, and Streptococcus mucosus capsulatus by others. It has been isolated by us from eight cases of pneumonia, from two cases of cold, and from two normal individuals, and has been seen in mixed cultures in a number of other cases. Three of the cases of pneumonia were early autopsy cases. In two of these the organism occurred pure and in large numbers (two hundred colonies were fished in one case and the resulting cultures were all similar); in the third case it was accompanied by a smaller number of the first variety of typical pnuemococci. In one pneumonic sputum and in one normal individual the first variety of typical pneumococci also accompanied it, while in all the other cases it was the only pneumococcus-like organism isolated. It has thus been found by us more frequently in

cases of pneumonia than in other cases. We have classed it among the typical pneumococci for the following reasons:

1. On serum-free culture media after the first two or three culture generations it produces no mucous-like material and shows no capsule or chain formation, but appears like a typical pneumococcus.

2. It readily coagulates the Hiss inulin medium.

3. It shows very distinct capsules in serum media and in the blood of inoculated animals.

4. It has been found pure and in large numbers in two cases of typical lobar pneumonia.

5. The results obtained from absorption experiments (see the Collins report) indicate a close relationship between it and certain typical pneumococci of the second variety, while no relationship is shown between it and the strains of typical streptococci tested.

It has been classed with the streptococci heretofore because of its ability readily to produce rounded forms and short chains. According to our descriptions of typical and atypical pneumococci it might be classed by many with the latter, but considering its ability under certain conditions to show typical pneumococcus forms we prefer to class it with the former, and make it a distinct variety. With regard to nomenclature, it should be called, according to the classification followed, Streptococcus lanceolatus, var. mucosus (the classification of Lehmann and Neumann, which we prefer), or Diplococcus lanceolatus, var. mucosus (the classification of other authors); we have given it the trivial name, Pneumococcus mucosus.

By refering to the section on serum reactions below and to Dr. Collins's report on the agglutination of the pneumococcus, it will be found that all the strains of this variety isolated by us show a specific similarity in these reactions.

A certain number of cultures from both normal and abnormal cases, which showed the characteristics of typical pneumococci immediately after isolation, have later dropped some of these characteristics and become more like streptococci. They appear principally in chains and no longer coagulate the inulin-serum medium. Whether some of these cultures were mixed in the

beginning with a streptococcus-like organism growing in intimate connection with the pneumococcus, as the influenza bacillus does, and finally outgrowing it, or whether they are all mutating varieties, is still a question. With such a mass of cultures it was impossible to follow each closely, to make plates, and to study colonies of each new culture generation, but, judging from the few apparently changing strains which have been more minutely studied, it would seem as if some of these cultures were really changing by mutation. None of them have become permanently typical streptococci—that is, they show more or less irregularity in chain production, sometimes produce elongated and pointed twos and always green color in blood-agar plates, but they seem gradually to lose their power to coagulate inulin-serum medium. These observations in regard to mutating varieties indicate a close relationship between certain pneumococci and streptococci, a relationship which previous investigators have noted.

All strains of pneumococci tried coagulated, usually within forty-eight hours, serum media containing dextrose, lactose, or saccharose, as do also certain strains of streptococci. With mannit different strains of pneumococci act differently. Out of one hundred strains tested, twenty-nine did not coagulate mannit-serum medium after fourteen days. Among the seventy-one coagulators, sixteen coagulated in twenty-four hours, seventeen in forty-eight hours, one on the third day, five on the fifth day, and the rest between the fifth and fourteenth days. With the exception of the mucosus variety, there seems to be no relation between this coagulation and the varieties or groups of pneumococci. All of the Pneumococcus mucosus strains tested coagulated the mannit medium within two days. Certain atypical strains which did not coagulate the inulin readily coagulated the mannit medium, while the few definite streptococci tried did not coagulate either. The plate growths from these non-coagulating cultures all showed practically as many colonies as those from the coagulating ones.

Virulence.—The virulence of the different strains of pneumococci for lower animals depends in great measure upon the method of isolation used. If the plate method be employed, fishing individual colonies, the majority of pure cultures ob-

tained will be distinctly less virulent than those isolated by the mass-culture method. The mass-culture method consists in inoculating a mass of sputum or material to be tested into serum-broth (previously tested for ability to give abundant growth of pneumococci), placing at 36° C. for twenty-four hours, and inoculating a certain amount of the resulting culture subcutaneously into the animal chosen. The culture isolated from the heart's blood of the animal at autopsy is then tested for virulence in the same species of animal.

We have used both rabbits and white mice for the inoculations, but in the great majority of cases the former animals only. Young rabbits, weighing from 800 to 1000 grams, and young adult mice have been chosen.

By testing the virulence of strains isolated by the mass-culture method, it has been shown that the percentage of virulent strains of pneumococci isolated from cases of pneumonia is higher than the percentage of those isolated from normal cases (see Table IV).

TABLE IV.

PERCENTAGE OF VIRULENT STRAINS.

Amount Inoculated.	Pneumonia Cases.	Healthy Individuals.
4.0 c.c.	87 %	69 %
0.1 c.c.	51 %	31 %

Most of the strains isolated from the cases of broncho-pneumonia which are included with the cases of pneumonia are not very virulent, while most of the strains from the colds which have not been noted here are virulent. Among the normal individuals the largest percentage of virulent pneumococci came from the Foundling Hospital children, the next from the Bellevue students, the next from the country around New York, and the smallest from Saranac Lake. Too much weight should not be attached to this summary, because of the comparatively small number of cases examined.

Normal No. 40 in contact with Pneumonia No. 36 were of equally extreme virulence for both mice and rabbits. All of the

Pneumococcus mucosus strains tested have been with one exception extremely virulent for mice and decidedly less so for rabbits.

Retention of Virulence.—Grown on artificial media, all of the virulent strains are losing their virulence although those transplanted on media containing blood from the species of animal used for the testing remained more virulent longer, for that species than for the other species of test animal chosen. No. 36, however, one of the most virulent strains tested, remained virulent for a long time for both rabbits and mice when grown on rabbit blood-agar. It seems now gradually to be losing its virulence for both animals. When grown in Bolduan's calcium-broth medium (*New York Med. Jour.*, May, 1905), cultures of pneumococcus remain alive and retain their virulence as long as when grown in serum-broth according to the few tests made; therefore, as this medium generally allows an abundant growth, it is an excellent one to use when for any reason the use of serum is undesirable.

Agglutination reactions are described in a separate report by Dr. Collins.

SERUM REACTIONS.

Specific Protective Substances.—According to Neufeld and Rimpau (*Deutsche med. Woch.*, 1904, p. 1458), the serum of rabbits inoculated with pneumococcus cultures becomes speedily protective for white mice. They claim to have obtained after the second inoculation of large doses of pneumococcus bodies, the first killed by heat and the second living, a serum which was highly protective for mice. They claim that this serum has no lytic properties for the pneumococcus, but that the specific protective substance is a bacteriotropic substance uniting with the bacteria and preparing them for ingestion by the leucocytes, and that when this serum is added to a mixture of bacteria and normal leucocytes in vitro more phagocytosis is produced than when normal serum is used. So far Neufeld's bacteriotropic substance agrees with Wright's (*Proceedings of the Royal Soc. of London*, 1903, LXXII, 337) opsonic substance, except that Neufeld claims that his substance is not destroyed by low heat, while Wright says that his is. Therefore Neufeld states that his bacteriotropic substance is not the same as Wright's opsonic substance.

Very little as yet has been done by us in attempting to raise in animals specific protective substances for the pneumococcus or in studying the properties of such substances. In the beginning we followed Neufeld's method, inoculating large doses of surface cultures of pneumococci into rabbits. The first cultures were subjected to from 60° to 65° C. for from fifteen to thirty minutes and the subsequent cultures were living. There is no doubt that a preliminary large dose of a dead culture could be followed by a larger dose of a living culture without causing death than if a small preliminary dose had been used, but the serum of such rabbits showed no protective action in mice with any of the strains of pneumococcus tested. Only a few tests were made, however, so no definite conclusion can be drawn. The phagocytic power in vitro seemed to be slightly increased for some of the strains, each by its own serum.

It was found that the opsonic power of normal rabbit, sheep, and especially of normal horse serum is very great for some strains of pneumococci, less so for others, and very slight for others. All of the strains of Pneumococcus mucosus tested belong to this last group. Since rabbits proved unsatisfactory, it was decided to experiment with sheep. Two sheep were chosen, one of which was inoculated with one of the first variety of pneumococcus and the other with a strain of Pneumococcus mucosus. The sheep have received eleven inoculations and have been bled twelve times. (See Table V.)

The serum from each bleeding was tested in vitro for its opsonic or bacteriotropic power on a number of strains of pneumococci, and from a few of the bleedings it was tested in addition for its protective power in white mice. In testing the opsonic power of the serum in vitro the following technic was used: To 0.5 c.c of serum, undiluted or diluted, in a short wide test tube, were added 0.5 c.c. of a thick suspension of washed normal leucocytes and 0.5 c.c. of the dilution of bacteria. The washings and dilutions were made with 0.8 % of sodium chloride solution. The mixtures were kept at 36° C. and smears made at stated times. The leucocytes almost immediately form a thin layer about the sides and bottom of the test tube and a well spread smear

containing large numbers of leucocytes is made by scraping from this layer with a flatly coiled platinum loop and spreading quickly on a clean glass slide. The smears were fixed in methyl alcohol and stained with eosin and methylene blue. Normal leucocytes from rabbits, guinea-pigs, sheep, and horses have been used, and so far our results have agreed with those of all other

TABLE V.

INOCULATIONS AND BLEEDINGS OF SHEEP.

Amount Inoculated.	Date of Inoculation.	Date of Bleeding.
20 c.c. of 24-hr. broth cult. centrifugalized and exposed to 60° C. for 20 min.	March 3	—
27 c.c. of 24-hr. broth cult. centrifugalized and exposed to 60° C. for 20 min.	March 10	March 15
28 c.c. of 24-hr. broth cult. centrifugalized and exposed to 60° C. for 10 min.	" 17	" 24
32 c.c. of 24-hr. calcium-broth cult. centrifugalized	" 29	April 2
40 c.c. of 24-hr. calcium-broth cult., 30 c.c. centrifugalized and 10 c.c. non-centrifugalized	April 8	—
50 c.c. of 24-hr. calcium-broth cult., 30 c.c. centrifugalized and 20 c.c. non-centrifugalized	" 19	April 27
60 c.c. of 24-hr. calcium-broth cult., 30 c.c. centrifugalized and 30 c.c. non-centrifugalized	" 28	May 4
70 c.c. of 24-hr. calcium-broth cult., 30 c.c. centrifugalized and 40 c.c. non-centrifugalized	May 5	" 10
80 c.c. of 24-hr. glucose-calcium-broth cult., 30 c.c. centrifugalized and 50 c.c. non-centrifugalized	" 15	" 24
85 c.c. of 24-hr. glucose-calcium-broth cult., 30 c.c. centrifugalized and 55 c.c. non-centrifugalized	" 27	June 5
50 c.c. of 24-hr. glucose-calcium-broth cult., +10 slant blood-agar cult.	June 8	" 15
	—	" 21

observers in regard to the indifferent action of leucocytes from different species of animals. According to our experiments, some species of leucocytes need more careful washing than others, probably because of the greater opsonic power of the corresponding normal serum. For example, horse leucocytes must be most carefully washed in order to keep the controls from showing phagocytosis. We have used horse leucocytes for many of the experiments because of our ability to obtain them easily and quickly in large quantities. The horse is bled just before the leucocytes are to be used and the blood is collected aseptically in flasks, with one tenth its volume of a 10 % solution of sodium

citrate in normal salt solution. After mixing, the blood is allowed to stand, and within ten minutes the red blood cells have settled, leaving the plasma, containing many leucocytes, above. This is drawn off, centrifugalized, and the leucocytes are washed carefully four times; each time fresh sterile plugs are used for the tubes. In this way it is easy to obtain a large amount of a very thick suspension of actively motile polynuclear leucocytes. Of such a suspension 0.5 c.c. added to the mixture of 0.5 c.c. each of 0.8 % salt solution and the required dilution of bacteria has been used as one control, and a similar mixture with normal serum in the place of the 0.8 % salt solution as another.

The dilutions of the bacteria were prepared as follows: A twenty-four-hour calcium-broth culture made from a twenty-four-hour blood agar-slant culture of the stock culture (the blood-agar was made from rabbit blood and kept in the thermostat at 36° C. for two days before using) was centrifugalized and enough 0.8 % salt solution added to the bacteria to make a suspension of about 2,000,000,000 bacteria to the cubic centimeter.

In estimating the phagocytic action by this method, it has been found that a large number of polynuclear leucocytes must be counted, as phagocytosis seems to occur irregularly, a group of polynuclear leucocytes each one loaded with bacteria filling one field, and a group containing no organisms the next.

The mixtures were examined in the beginning, at the end of one quarter, one half, two, three, five, and twenty-four hours. It was found that the difference between the serum controls or heated serum and the specific serum was more marked after fifteen to thirty minutes than at the height of phagocytosis, which occurred in from two to three hours. At the latter time the differences, if any, were very slight. The specific serum thus seemed to allow the phagocytosis to occur more quickly.

The difference between the opsonic power in vitro of the normal serum and the specific serum, however, has so far been slight. This slight increase of opsonic power of the specific serum was apparent after the second bleeding and continued up to and including the eighth bleeding, but the serum from the next two bleedings (ninth and tenth) showed no definite difference in

phagocytosis between normal serum controls and specific serum. The serum from the ninth bleeding, however, showed a protective power for mice similar to that of the serum from the eighth bleeding, and the serum from the tenth bleeding prolonged life. As the control animals in these experiments all died, the absolute protective power of these sera is not known. From these data all that can be said is that while the phagocytic power in vitro of a certain specific serum seemed no greater than that of the control serum, yet the former possessed marked protective power in mice. One of the heterologous strains (Pn. 4) showed clumping and marked phagocytosis with Sheep Serum II (inoculated with Pneumococcus mucosus), while with Sheep Serum I (inoculated with Pn. 36) it showed no clumping and less phagocytosis, and yet mice were protected from it by this latter serum. All of the Pneumococcus mucosus strains showed very slight phagocytosis in any serum, and yet with Sheep Serum II mice were protected from all of the strains with but one exception.

It seems from these observations that the degree of phagocytosis in vitro with some sera at least is not an indication of the degree of protective power in mice.

In regard to the influence of heat upon the phagocytic power of these sera, the following results have been obtained: 60° C. for a half hour has slight deleterious effect, 65° for twenty minutes has more, and 60° for one hour has a marked effect.

Poured blood-agar plates after two hours at 36° C. show a decrease in the number of colonies with all the strains which agglutinated, but the decrease is no greater than could probably be accounted for by the agglutination.

SUMMARY AND CONCLUSIONS.

1. Typical pneumococci were present during the winter months in the throat secretions of a large percentage of healthy individuals in city and country.

2. A higher percentage of atypical strains of pneumococci have been obtained from healthy persons than from those suffering from pneumonia. In the latter cases the atypical strains may have been overlooked, because of the larger number of typical

pneumococci present. Many of the atypical strains seem to be closely related to the streptococci.

3. The so-called Streptococcus mucosus Schottmüller, which has hitherto been classed with the distinct streptococci, is placed as a definite variety among the pneumococci, and it is recommended that the name be changed to Streptococcus lanceolatus, var. mucosus.

4. A lower percentage of strains of pneumococci virulent for rabbits in the doses used has been obtained from normal cases by rabbit inoculations of mass cultures than from cases of pneumonia by the same method.

5. Since the virulence of pneumococci may be rapidly increased for a susceptible species of experimental animal by successive passage, and since pneumococci obtained from most pneumonias are more virulent for experimental animals than are those obtained from healthy individuals, therefore the virulence of pneumococci from cases of human infection is probably increased for human beings; hence cases of pneumonia should be considered to a certain degree as contagious and, since the virulence of the pneumococcus may be quickly increased and since the organism is very prevalent in normal sputum, all possible measures should be taken to restrict public expectoration.

6. By repeated inoculations into sheep of a pneumococcus strain, a specific protective power of this serum for mice is developed against the homologous strain and against certain other strains, one morphological variety (Streptococcus lanceolatus, var. mucosus) being thus clearly differentiated from other strains.

7. Coincident with this production of protective power, a slight specific increase of the sheep serum in phagocytic power in vitro has been observed with some strains of pneumococci, all strains of Streptococcus lanceolatus, var. mucosus, acting similarly with the serum produced by the inoculation of one strain; the strains of some other varieties, however, have shown no definite relationship between the phagocytic power and the protective power of the serum.

Note.—The protocols of cases and experiments will be published later in the Reports of the Health Department of New York City.

THE APPLICATION OF THE REACTION OF AGGLUTINATION TO THE PNEUMOCOCCUS.

By KATHERINE R. COLLINS, M.D.,

Assistant Bacteriologist.

(*From the Research Laboratory of the Department of Health, New York City.*)

The following report is a part of the work on pneumonia as planned by the Commission for the Investigation of Acute Respiratory Diseases, and by the Department of Health.

Normal serum of various animals differs greatly in its tendency to agglutinate many strains of pneumococci. Thus rabbit serum generally gave negative results, while sheep and horse serum reacted slightly in a few instances, and the serum of one goat of four tested agglutinated a number of strains of pneumococci in dilutions of 1 : 10.

Of two normal human sera tested, one failed to react while the other agglutinated several of the organisms in dilutions as high as 1 : 10.

Neufeld, Clairmont, Landsteiner, Wadsworth, Heyrovsky, and others have succeeded in producing agglutinins for pneumococci in the animal body through immunization.

Gorgáno and Fattori state that agglutination of Diplococcus pneumoniæ with the blood of patients suffering from infection with this organism is constant, that it persists for some time after recovery of the patient, and that the reaction is more marked if the homologous organism be used. The highest reaction obtained by them was, however, in a 1 : 10 dilution.

Glucose broth has been generally recommended as the medium best adapted for making agglutination tests with the pneumococcus, but as the organism quickly dies out in the presence of the excess of acid produced by the fermentation of the sugar, it can be carried through one generation only on this medium,

unless it is transferred before the acidity has increased sufficiently to destroy the growth. This fact makes the broth unsuitable for the work.

Marshall and Knox [1] and Morello have shown that the typhoid bacillus loses its agglutinability when grown for some time in an active immune serum. Dr. Park and I have demonstrated the same to be true for the bacillus of dysentery when grown in its immune serum, and we were also able to show, further, that the agglutinability could be restored by long cultivation upon suitable media.

As the presence of serum constituents in the medium is required for the continuous growth of the pneumococcus, it might be assumed from the above facts that the agglutinability of the organism might at least be lowered by long cultivation upon a medium containing even very small amounts of these inhibitory substances. This assumption was borne out by several tests made with an organism taken on the one hand directly from a fresh rabbit-blood-agar culture, and on the other from a culture in calcium broth [2] one generation old and several generations old. The last culture gave the best reaction, while the culture from the blood-agar gave the poorest reaction.

To eliminate this source of error, diluted sheep or hog serum, as suggested by Dr. Park, was boiled to destroy any inhibitory substances present, and added to broth or agar as the case required. Cultures obtained from media containing these sera, when transferred to calcium broth, usually gave a homogeneous growth with much less tendency to spontaneous agglutination than is seen with cultures grown in calcium-glucose broth. Heating the organism to 70° C. for 15 minutes does not affect its agglutinability. Heating the serum to 85° for 15 minutes,

[1] The studies of Marshall and Knox, so far as I know, have not as yet been published.

[2] The fact that the addition of calcium carbonate to culture media neutralizes the acid formed by the fermentation of sugar during bacterial growth has been recognized for some time. The application of this reaction to the growth of the pneumococcus was suggested independently by Bolduan and Hiss. The former recommended the addition of bits of marble to plain broth, the latter used calcium carbonate in the form of powder in glucose broth.

however, destroys the agglutinins both for the pneumococcus and the Pneumococcus mucosus.[3]

Several methods of immunization were tried. The one which gave the best results is represented by the following example:

Feb. 15. A rabbit was given subcutaneously 3 c.c. of a culture grown for 48 hours in broth to which a few drops of defibrinated blood were added, heated previously to 60° C. for 30 minutes.
Feb. 28. 5 c.c. of a similar culture administered.
March 8 ½ c.c. of a living culture of the same organism was given.
" 13 1 c.c. " " " " " " " " " "
" 24 5 c.c. of a calcium-broth culture heated 70° C. for 15 minutes given.
April 1 5 c.c. " " " " " " " " " "
" 8 5 c.c. " " " " " " " " " "
" 15 10 c.c. " " " " " " " " " "
" 25 10 c.c. " " " " " " " " " "
May 1 10 c.c. " " " " " " " " " "
" 8 15 c.c. " " " " " " " " " "
" 15 15 c.c. " " " " " " " " " "
" 21 Animal bled and the serum tested with the homologous organism which it agglutinated in a dilution of 1:200.
May 22 An emulsion heated to 70° C. for 15 minutes from four heated serum-agar plates was injected subcutaneously. Animal dead on the following day.

Hanging drops were chiefly relied on for ascertaining the reactions, though in many instances these were controlled by the macroscopic method and the contents of the tubes examined microscopically after reaction had taken place.

The sources of error seem about equal in the two methods, while the hanging drop has the advantage of shorter time limit of reaction, and of easy recognition of contamination.

With the pneumococcus the tube method generally indicates a higher microscopical reaction than the hanging drop. This is contrary to tests made with the dysentery and typhoid bacilli, and is explained by the fact that in the former case the free organisms must be present in great numbers to cloud the supernatant fluid, whereas in the latter a comparatively small number of free bacilli may render the fluid turbid, so that in the case of the pneumococcus a good reaction viewed macroscopically may become only a fair reaction when viewed microscopically.

[3] A description of this organism will be found in the article by Drs. Park and Williams in this volume.

Neufeld states that the various strains of the pneumococcus agglutinate alike, an observation probably due to the low reactions obtained by him, since his maximum reaction was 1 : 50. My work has shown great irregularity in this respect, the serum of an animal immunized with one strain of pneumococcus agglutinating only seven organisms out of seventy tested in dilutions equalling the reaction (1 : 200) with the homologous organism. Four strains reacted in dilutions of 1 : 10, eleven in 1 : 2, while the remaining organisms were entirely negative.

The serum of a second immunized animal agglutinated the homologous organism in dilution of 1 : 100, while other strains were affected in less dilutions or not at all.

TABLE I.

AGGLUTINATION TESTS WITH THE SERUM OF A SHEEP[4] INJECTED FOR A PERIOD OF THREE MONTHS WITH PNEUMOCOCCUS NO. 36.

Dilution.	2	10	20	50	100	Control.
Typical pneumococcus 36.....	++	++	++	++	+1	—
" " 14.....	—	—				—
" " 4.....	++	1				1
" " 16.....	++	—				—
" " 18.....	—					—
" " 33.....	—					1
Atypical " 2.....	++	—				—
" " 66.....	+1	—				1
Pneumococcus mucosus 47.....	—					—

The remaining sixty-one organisms tested reacted some in dilution of 1 : 2 and others not at all, thus emphasizing the distinction of pneumococcus No. 36 from the other strains in regard to its power to produce agglutinins.

[4] For the details of the immunization of the sheep, the paper of Drs. Park and Williams is to be consulted.

There is apparently a difference in the agglutinability of the pneumococci, some strains uniformly reacting much more readily than others in normal and immune sera.

A rather interesting fact that deserves further investigation is this: Two strains of pneumococci which showed good agglutination with several active sera failed to produce agglutinins to any extent in the animal body for themselves or other strains of pneumococci. A rabbit and a goat were inoculated without results with one strain, and a rabbit and a horse with the other; and as the same kind of animals was immunized under the same conditions with other strains with good results, this irregularity would seem to indicate a peculiarity of the organism rather than of the animals injected.

Another observation of interest, but one which has not been carried far enough on account of insufficient time to establish definite conclusions, is certain reactions obtained with a streptococcus serum and absorption experiments made with this organism.

A young goat which was immunized with a strain of streptococcus yielded a serum which agglutinated a few pneumococci and its own culture in dilutions of 1 : 10.

Pneumococcus 66, which coagulates inulin-serum water late, absorbs the agglutinins for several pneumococci from a typical pneumococcus immune serum. This culture is the only one of the pneumococci that has its agglutinins taken out of the above streptococcus immune serum by the streptococcus; it produces agglutinins in the animal body for itself and many of the pneumococci. These reactions suggest the possibility of the occurrence of intermediate types of organisms between pneumococci and streptococci.

EXHAUSTION EXPERIMENTS.

The extreme sensitiveness of the pneumococcus to changes of conditions not readily determined brings about variation in the behavior of this organism which proved a serious factor in the application of the agglutination reaction and in the interpretation of the results obtained. To eliminate as far as possible

any errors arising from this instability, the exhaustion experiments were conducted in groups, each group covering as many observations as practical, in order to insure uniform conditions for a number of tests.

In the exhaustion experiments a slight loss of agglutinins has generally been observed. This loss occurs whether the organism used for absorption is an homologous or a related one or of a foreign type. This fact points to the cause of the loss lying outside of the presence of the organism. The loss is readily estimated on account of its uniformity, and in no way affects the determination of the amount of absorption excepting where a strain reacts only in low dilutions. In this case the disappearance of the agglutinins cannot be ascribed with certainty to the organism used for absorption, and the establishment of relationship by the absorption method is not possible in these instances.

Testing the reaction of the meningitis coccus in antipneumococcus serum, Sorgente failed to obtain agglutination with a number of strains. We failed to absorb the agglutinins from a serum agglutinating several strains of pneumococci in dilution of 1:200 with a culture of the diplococcus of meningitis.

As shown in Table II, the power of the serum to agglutinate pneumococcus Nos. 14 and 72 in equally high dilutions with the homologous organism, and by absorption that the agglutinins are group agglutinins in the case of the former, and both group and specific agglutinins in the case of the latter organisms, are readily explained by the fact that the two types of agglutinins constantly vary in ratio both in different animals and at different periods of inoculation, the group agglutinins exceeding even at times the specific ones.

The increase of agglutinins for different strains of Pneumococcus mucosus in the serum of an animal inoculated with one of the typical pneumococcus strains, and the results obtained by the absorption of these agglutinins, separate them into a distinct variety from the majority of other pneumococci.

The similar results obtained, as indicated in the following table, by absorption with Pneumococcus mucosus No. 47 and

TABLE II.

SERUM OF A SHEEP INJECTED WITH PNEUMOCOCCUS MUCOSUS (WILLIAMS), SHOWING THE AGGLUTINATION INDEX BEFORE AND AFTER ABSORPTION WITH ITS OWN GROUP, TYPICAL PNEUMOCOCCI, A STRAIN OF STREPTOCOCCUS, AND BACILLUS TYPHOSUS, RESPECTIVELY.

		Before Exhaustion.								After Exhaustion with its Own Type.		After Exhaustion with Pneumococcus No. 14.				After Exhaustion with Pneumococcus No. 4.		After Exhaustion with Streptococcus longus.					After Exhaustion with B. typhosus.			
			6	10	20	50	100	200	Control.	6	20	6	20	50	100	6	20	6	20	50	100	200	6	20	50	100
Pneumococcus mucosus.	25	++	++	++	++	++	++	+1	−+	−+	−+	++	++	++	−+	−+	−+	++	++	++	++	−+	++	++	+1	+
	47	++	++	++	++	++	++	++	−	−	−	++	++	++	+	1	1	++	++	++	++	+	++	++	++	+1
	C1	++	++	++	++	++	+1	−	−	+	−	++	++	+1	−+	−+	−+	++	++	+1	+	−+	++	++	+1	+
	C4	++	++	++	++	++	++	++	−+	−+	−+	++	++	+1	+	−	−	++	++	+1	+	−+	++	++	++	−+
Atypical Pneumococci.	2	++	++	++	+				−	++	+	++	++	+	1	−	−	++	++	−+	1		++	++	+1	
	72	++	++	++	+				1	+	−	+	−+	1		−+	−+	++	++	++	1		++	++	+	
Typical Pneumococci.	4	++	++	++	++	++	+1	+	−+	++	−+	−+	−+	−+		−	−	++	++	++	+		++	++	+1	2
	14	++	++	++	++	++	++	+	−	1	1	−+	1			1	1	++	++	++	+		++	++	++	1
	11	−+							−																	
	36	−							−																	
	56	1							1																	
Typical Pneumococci. Simple Cold.	2																									
	C.& D.	1							1																	
Normal.	N. 46	+1	+	−+					1																	

Part of the remaining organisms tested reacted in dilutions of 1:2 and part failed to react even in this low dilution.

++complete agglutination; +1 not quite complete; +good; 1 trace; −+fair; −negative.

TABLE III.

		Serum of a goat, injected during a period of three months with pneumococcus No. 14, before and after exhaustion with pneumococcus No. 66 and streptococcus longus.															Serum of a horse injected during a period of three months with pneumococcus No. 14 before and after exhaustion with pneumococci Nos. 22 and 72.											
		Before Exhaustion.							After Exhaustion with Pneumococcus No. 66.				After Exhaustion with Streptococcus longus.					Before Exhaustion.					After Exhaustion with Pneumococcus No. 22.				After Exhaustion with Pneumococcus No. 72.		
			6	20	50	100	200	Control.	6	20	50	100	6	20	50	100	200	6	20	50	100	200	6	20	50	100	6	20	50
Typical Pneumococcus		4	++	++	++	+1		−	−				++	++	++	+		+	1				+	−			−		
"	"	14	++	++	++	++	+	−	−				++	++	++	+		++	++	+			1				1		
"	"	22	++	++	++	+		−	++	++	−+		++	++	+1			++	++	+	+		1	1			−+	−+	
"	" Normal	46	++	++	++	++	+1	−	+1	+1			+1	+1	−+			++	++	++	++	+1	+1	+			+1	+1	
Atypical	"	66	++	++	++	+1	−+	−+	−+				−+	−+				++	++	−+	−+		++	++	−+		−+	−+	
"	"	2	++	++	++	+1		−	+	−			++	++	++	−+		++	−+				++	−			+1	−+	
"	"	72	++	++	++	++	+	1	++	++	1		++	++	++	+1		++	++	++			++	++	++	1	1		

The remaining organisms tested reacted as in Table II. The above tests were made simultaneously, the same cultures being used throughout.

The irregularity of the pneumococci in their behavior to the agglutination reaction is amply indicated by the tables. The interaction of typical and atypical cultures does not bear out the classification based upon the morphological and cultural characteristics of the organisms. Atypical pneumococci 2, 66, and 72 differ constantly from each other, and yet correspond with several of the typical pneumococci in their agglutination reactions.

The different degrees of partial exhaustion of agglutinins for normal culture No. 46 suggest the existence of agglutinins common to this culture and several other strains of pneumococci, as well as to the Streptococcus longus.

pneumococcus No. 4 (the latter organism when studied not producing the characteristic capsule and gelatinous colonies), suggest that these cultural attributes may be lost while the agglutinative affinity is still retained.

The ability of the Pneumococcus mucosus group to produce common agglutinins for some pneumococci, and the fact that the streptococcus failed to affect through absorption their agglutinins, would indicate a closer relation of this variety to the pneumococci than to the streptococci.

CONCLUSIONS.

Owing to unavoidable circumstances only a limited amount of time was available for the work on agglutination. The foregoing report is therefore preliminary only and the following conclusions are provisionally offered:

I. Pneumococci by reason of their agglutinating properties exhibit a tendency to separate into numerous groups similar to streptococci.

II. Pneumococcus mucosus forms a distinct and consistent variety. The production by it of common agglutinins for some pneumococci and the resistance of the agglutinins produced by it to absorption by the streptococcus indicate a nearer relation to the former than to the latter organism.

III. The agglutinating substances in the serum of immunized animals were demonstrated by absorption tests to consist of specific and group agglutinins in cases where the agglutinins were sufficiently developed to make use of this method.

IV. The pneumococci seem to show marked differences in their ability to undergo agglutination.

V. There was considerable uniformity of reaction of the various strains in low dilutions, but this uniformity is not continued as the animal becomes more highly immunized.

VI. At present it is not possible to establish a definite relation between the agglutination reaction and the other characteristics of the pneumococcus excepting in the case of the Pneumococcus mucosus.

The above work has been conducted under the direction of Dr. Wm. H. Park, Director of the Research Laboratory of the Department of Health of New York City, to whom I desire to express my appreciation of the interest he has shown in the work.

REFERENCES.

Bolduan.—*New York Medical Journal*, 1905, lxxxi, 956.
Clairmont.—*Zeitschrift für Hygiene und Infektionskrankheiten*, 1902, xxxix, 1.
Heyrovsky.—*Centralblatt f. Bakteriologie u. Parasitenkunde*, 1905, xxxviii, 704.
Hiss.—*Journal of Experimental Medicine*, 1905, vii, 223.
Landsteiner.—*Wiener klinische Wochenschrift*, 1897, x, 439.
Morello.—*Annali d'Igiene Sperimentale*, 1904, xiv, 153.
Neufeld.—*Zeitschrift für Hygiene und Infektionskrankheiten*, 1902, xl, 54.
Park and Collins.—*Journal of Medical Research*, 1904, vii, 491.
Sorgente.—*Centralblatt f. Bakteriologie u. Parasitenkunde*, 1905, xxxix, 1.
Wadsworth.—*Journal of Medical Research*, 1903, x, 228.

A COMPARATIVE STUDY OF PNEUMOCOCCI AND STREPTOCOCCI FROM THE MOUTHS OF HEALTHY INDIVIDUALS AND FROM PATHOLOGICAL CONDITIONS.

By WARFIELD T. LONGCOPE, M.D.,

Director of the Ayer Clinical Laboratory of the Pennsylvania Hospital, Philadelphia,

and W. W. FOX, M.D.

From the 1st of November, 1904, until the 1st of May, 1905, there were studied sixty-nine strains of organisms which appeared to belong to the groups of streptococci and pneumococci. The morphological, biological, and pathogenic properties of these organisms were investigated. For convenience of description and study all these bacteria were grouped into three main classes, Series A, Series B, and Series C.

Series A comprised all the organisms isolated from the saliva of healthy individuals, by the inoculation of the saliva into mice and rabbits, by making streak plates from the buccal secretion, or by both methods.

Series B included all the organisms obtained from pathological conditions, which from their cultural characteristics and effects upon animals were thought to be pneumoccoci.

Series C included all the organisms obtained from pathological conditions which from appropriate tests were considered as streptococci.

METHODS.

In general the method of procedure was to study the morphology of the organisms by cover-slip preparations from the original fluid or organ from which they were cultivated. Three varieties of culture medium at least were employed—blood-agar, plain agar, and blood-serum,—and the morphology was studied in the cultures and the exudates and body fluids of the inoculated

animals. As a routine the organisms were grown upon blood-agar, plain agar, bouillon, litmus milk, inulin-serum water, potato, gelatine, and unheated human blood-serum. The media were all standardized by titration with $\frac{1}{10}$ N. sodium hydroxide and made neutral to phenolphthaleine. It may be said that the only media which proved of great practical value in differentiating the various strains of cocci were inulin-serum water, blood-serum, and blood-agar. The blood-agar was of great service as a medium for preserving stock cultures. It was made by the addition of a few drops of defibrinated human blood to a tube of melted agar. Organisms grown upon this medium, in tubes covered with a rubber cap, remained viable for several weeks when kept in the dark at 0° to 10° C. Moreover, their virulence was well retained. One culture of Series B of pneumococci after a month's growth on blood-agar killed rabbits in doses of .0001 c.c. of a twenty-four-hour bouillon culture, which was the original minimal fatal dose.

Finally, the virulence of the organisms was tested for rabbits and mice, and the lesions produced in these animals studied bacteriologically and frequently histologically.

Series B may first be considered; it comprises sixteen organisms. The following table gives the main data concerning their growth and pathogenic properties.

TABLE I.

Series B.

No. of Organism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
II.	Blood culture, lobar pneumonia.	11/4/04	Lanceolate diplococci, no chains. Gram positive.	Positive; blood-agar.	48 hrs., clot.	0.5 c.c. 24-hr. bouillon culture subcutaneously does not kill mice. 1 c.c. 24-hr. bouillon culture subcutaneously kills rabbit, 760 grams, in 18 days; much emaciation; no local lesions. Cultures from heart and peritoneum sterile. One blood-agar slant intraperitoneally kills rabbit, wt. 1020 grams, in 36 hrs. Fibrino-purulent peritonitis; large hard spleen; cultures, heart and peritoneum, positive.

TABLE I (*Continued*).

No. of Organism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
III.	Culture from consolidated lung, lobar pneumonia.	11/6	Lanceolate diplococci, no chains. Gram positive.	Blood-agar; positive (ordinary stains).	24 hrs., clot.	4 c.c. 24-hr. bouillon cultur subcutaneously kills rabbit wt. 708 grams, in 5 days Extensive sero-fibrinous sub cutaneous exudate; broncho pneumonia; spleen large an soft. Cultures from hear positive. 2 c.c 24-hr. bouillo culture kills mouse intra peritoneally in 12 hrs.
IV.	Meninges, cerebro-spinal meningitis.	11/8	Lanceolate diplococci, no chains. Gram positive.	Blood-agar; positive (ordinary stains).	48 hrs., clot.	2 c.c. 24-hr. bouillon cultur intravenously kills rabbit wt. 374 grams, in 12 hrs Spleen small and hard. Afte passage through 13 rabbits 0.001 c.c. 24-hr. bouillo culture kills rabbit, wt. 195 grams, in 36 hrs.; produce usually extensive fibrinou exudate in subcutaneous tis sues with hard friabl spleen, also fibrino-purulen peritonitis, septicæmia wit subserous hæmorrhages; onc infarction of spleen.
V.	Pleura, lobar pneumonia.	11/15	Lanceolate diplococci, few short chains. Gram positive.		48 hrs., clot.	2 c.c. 24-hr. bouillon cultur subcutaneously, in rabbit, w 1300 grams, negative. 2 c.c 24-hr. bouillon culture in traperitoneally, in rabbit, w 1320 grams, negative. Deat in 35 days, no local lesions cultures negative.
VIII.	Lung, lobar pneumonia.	12/5	Lanceolate and round cocci in pairs. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	0.2 c.c. 24-hr. bouillon cu ture subcutaneously kil rabbit, wt. 1000 grams, i 12 hrs. Extensive sero-sa guino-fibrinous exudate i subcutaneous tissues; sma soft spleen. Cultures ar cover-slips from heart, su cutaneous tissues, and per toneum positive. 0.5 c.c. 24-hr. bouillon cultu kills second rabbit, wt. 15 grams, subcutaneously, 20 hrs. Slight subcutaneo œdema, small soft splee cultures from subcutaneo tissues, heart, and perit neum positive.

TABLE I (*Continued*).

No. of Organism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
IX.	Pus, empyæma.	12/10	Lanceolate and rounded cocci in pairs. Gram positive.	Positive; serum; blood-agar (Hiss).	48 hrs., clot.	0.4 c.c. 24-hr. bouillon culture subcutaneously, in rabbit, wt. 1480 grams, death in 12 days; extensive fibrinous exudate in subcutaneous tissues; large, hard spleen. Cultures from heart positive.
X.	Pus, empyæma.	12/13	Lanceolate cocci in pairs. Gram positive.	Positive; serum (Hiss).	No clot, 10 days.	2 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 1100 grams, death in 36 hrs. Extensive sero-fibrinous exudate, small soft spleen; cultures and coverslips positive. From subcutaneous tissues and heart, capsules readily stainable. One 24-hr. blood-agar culture subcutaneously in rabbit, wt. 1900 grams, death 3 days 12 hrs. Same lesions as above, with sero-fibrinous peritonitis.
XI.	Lung, lobar pneumonia.	12/17	Lanceolate cocci in pairs. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	One 24-hr. blood-agar culture subcutaneously in rabbit, wt. 1320 grams. Lives.
XII.	Otitis media.	1/14	Lanceolate cocci in pairs and short chains. Gram positive.	Positive; Blood-agar (ordinary stains).	48 hrs., clot.	One 24-hr. blood-agar culture subcutaneously in rabbit, wt. 820 grams; death in 7 days. Localized subcutaneous fibrinous exudate, fibrinous peritonitis, and pericarditis. Cultures from heart, peritoneum, and pericardium positive. Rabbit 3. 0.1 c.c. 24-hr. bouillon culture kills in 6 days. Cultures from heart and peritoneum positive.
XIII.	Lung, bronchopneumonia.	1/19	Lanceolate cocci in pairs. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 1400 grams; death in 10 days. Fibrinous subcutaneous exudate. Cultures from heart negative.

TABLE I (*Continued*).

No. of Organism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
XIV.	Pus, empyæma.	1/30	Lanceolate cocci in pairs and short chains.	Positive; serum (Hiss).	24 hrs., clot.	0.1 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 1500 grams; death in 2 days 12 hrs. Sero-purulent, peritonitis, pleurisy, and pericarditis. Small soft spleen. Cultures from heart positive. After passage in decreasing doses through 10 rabbits, 0.000,001 c.c. 36-hr. bouillon culture intraperitoneally in rabbit, wt. 1220 grams; death in 12 hrs. Lesions, sero-purulent exudate in serous cavities as a rule; spleen sometimes hard, sometimes soft; slight subcutaneous œdema; septicæmia always.
XVI.	Retro-pharyngeal abscess.	1/30	Round and lanceolate cocci in pairs. Gram positive.	Positive; serum (ordinary stains).	72 hrs., clot.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 988 grams; death in 19 days. Slight subcutaneous fibrinous exudate.
XVII.	Spinal fluid, meningitis.	2/20	Lanceolate cocci in pairs and short chains. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	1 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 580 grams; death in 3 days. Sero-fibrinous exudate in subcutaneous tissues. Cultures from heart and subcutaneous tissues positive. 3d rabbit. 0.01 c.c. 24-hr. bouillon culture subcutaneously, wt. 822 grams; death in 8 days. Sero-purulent peritonitis; culture negative.
XVIII.	Lung puncture, lobar pneumonia.	3/5	Lanceolate and oval cocci in pairs and long chains. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	1 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 1290 grams; death, 6 days. Subcutaneous fibrinous exudate, spleen hard, sero-fibrinous peritonitis. Cultures from heart and peritoneum positive. 0.5 c.c. 24-hr. bouillon culture subcutaneously does not kill rabbits.
XIX.	Sputum; same case as XVIII; taken at same time.	3/5	Lanceolate and round cocci in pairs. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	1 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 860 grams; death in 36 hrs. Subcutaneous congestion, œdema, and hæmorrhage; large, hard spleen. Cultures from heart positive.

TABLE I (*Continued*).

No. of Organism.	Source.	Date.	Mordhology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
XX.	Heart vegetations; acute endocarditis.	3/30	Lanceolate and oval cocci in pairs.	Positive; serum (Hiss).	48 hrs., clot.	0.5 c.c. 24-hr. bouillon culture intraperitoneally in 2d rabbit, wt. 1050 grams; death in 12 hrs. Moderate fibrino-purulent peritonitis; large, hard spleen. Cultures from heart and peritoneum positive.

The morphology was usually that of lanceolate diplococci; this was most constant in blood-agar, in serum, and in the exudates of animals. In other media chains of cocci were frequently noted, some made up of oval, almost round, or even slightly flattened elements. All stained positively by Gram's method of staining. Capsules could frequently be demonstrated by ordinary stains on organisms grown in serum, and without fail by the stains of Hiss. On blood-agar the growth was quite luxuriant, moist, raised, grayish, and often formed a thin watery film. There was always a marked tendency for the colonies to become confluent. The growth was heaviest and most typical with freshly isolated organisms. In blood-serum a very heavy cloud appeared in twenty-four to forty-eight hours, and large quantities of acid were formed. The serum in all these routine tests was taken from one patient suffering from chronic endocarditis who was bled copiously. In inulin-serum water all the organisms except one (10 B) produced acid and formed a solid clot in from twenty-four to seventy-two hours. This one organism produced perhaps faint traces of acid but no clot, even after ten days' growth. In all other respects it was identical with the other cocci of this group and unlike those grouped in Series C.

The pathogenicity of these bacteria varied greatly, from strains which even in large doses produced almost no effect in rabbits to strains which were highly virulent. No relationship could be traced between the source of the culture and the degree

of virulence. The most virulent cultures were obtained from pus from cases of empyæma, and from the cerebro-spinal fluid of cases of meningitis. Most of the cultures from the consolidated lungs of cases of lobar pneumonia showed a rather low grade of virulence. In five instances emulsions of consolidated lungs were inoculated into animals. The animals either did not die or lived for many days, and at autopsy cultures from the organs gave no results. In one instance (B III), cultures directly from the lung yielded a pneumococcus which in large doses killed rabbits. In two other instances streptococci (C VII and C XI) were recovered in cultures from the lungs. Of two organisms (B XVIII and B XIX) obtained in one instance from the saliva and from lung puncture in the same person suffering from acute lobar pneumonia, the coccus from the saliva proved the more virulent for rabbits. The pathological processes produced in the various animals varied greatly; no one organism gave a constant type of lesion. Extensive subcutaneous exudates of fibrin, fibrin and pus, and fibrin and serum were common. Fibrino-purulent peritonitis, pleurisy, pericarditis, and mediastinitis were often seen; septicæmia with and without hæmorrhages in the serous membranes and thymus gland were noted. In most instances the spleen was hard, friable, and showed on microscopic examination an extensive infiltration of fibrin with hyaline thrombi in the blood spaces. Occasionally it was soft and red. Both conditions were noted in animals inoculated at different times with the same organism. The liver was sometimes fatty, often showed cloudy swelling and congestion, and in some instances presented on microscopic examination foci of necrosis infiltrated with fibrin and leucocytes. The kidneys were often congested, and in some cases hyaline thrombi were observed in the glomerular capillaries. In one instance these had much the appearance of agglutinated red-blood-corpuscle thrombi. The heart was often flabby and distended with blood. The lungs were usually congested. In no instance was a pneumonia seen which could be ascribed to the action of the pneumococcus. A few animals became gradually emaciated and died after some weeks without local lesions. At autopsy cultures from the organs gave negative

results. In a few instances the animals developed sloughing sores of the abdominal wall from which they died after several days. Cultures usually showed a secondary invasion by another organism.

If these findings are compared with the table below, it will be seen how different are the two groups B and C.

TABLE II.

Series C.

No. of rganism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
I.	Blood culture, mastoiditis.	11/9	Small, round cocci in clusters and chains. Gram positive.	Negative; serum (Hiss).	No clot, 6 days.	Not tried.
II.	Arthritis, pus from joint.	11/14	Round and slightly lanceolate cocci in pairs and long chains. Gram positive.	Negative; serum (Hiss).	No clot, 7 days.	Not tried.
III.	Peritonitis, case of typhoid fever complicated with lobar pneumonia.	11/21	Round cocci singly, in pairs, and chains. Gram positive.	Negative; serum (Hiss).	No clot, 8 days.	2 c.c. 24-hr. blood bouillon culture intraperitoneally in rabbit, wt. 1050 grams; death in 21 days. Arthritis, left hind paw. Cultures contaminated.
IV.	Blood culture, puerperal sepsis.	11/25	Small, round cocci in pairs and chains. Gram positive.	Negative; serum (Hiss).	No clot, 6 days.	Not tried.
V.	Lung, acute broncho-pneumonia.	11/28	Small, round cocci singly and in pairs. Gram positive.	Negative; serum (Hiss).	No clot, 7 days.	Not tried.

TABLE II (*Continued*).

No. of Organism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
VI.	Pus from abscess.	12/31	Small, round cocci in pairs and chains.	Negative; serum (Hiss).	No clot, 7 days.	Not tried.
VII.	Lung, lobar pneumonia.	1/7	Small, round, and lanceolate cocci, singly, in pairs, and short chains.	Negative; serum (Hiss).	No clot, 6 days.	1 c.c. emulsion of lung subcutaneously in rabbit, wt. 1260 grams; death in 14 days. Subcutaneous abscess. One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 1090 grams; death, 21 days. Large circumscribed subcutaneous abscess. Culture from abscess positive.
VIII.	Culture from lung, terminal pneumonia.	1/11	Small, cocci in chains and clusters. Gram positive.	Negative; serum (Hiss).	No clot, 6 days.	Not tried.
IX.	Lung, lobar pneumonia.	3/29	Small, round, and flattened cocci in pairs and chains. Gram positive.	Negative; serum (Hiss).	No clot, 7 days.	One 24-hr. blood-agar slant intraperitoneally in rabbit, 800 grams; death in 24 days. Death due to septicæmia.
X.	Blood culture, empyæma.	4/13	Small, round, and slightly lanceolate diplococci in pairs and chains. Gram positive.	Negative; serum (Hiss).	No clot, 7 days.	5 c.c. original 24-hr. bouillon culture intraperitoneally in rabbit. Lived.
XI.	Lung, broncho-pneumonia.	4/20	Small, round, and lanceolate cocci in pairs and short chains. Gram imperfectly positive.	Negative; serum (Hiss).	No clot, 10 days.	2 c.c. emulsion of lung subcutaneously in rabbit, wt. 260 grams; death, 12 hrs. Extensive fibrino-purulent peritonitis. Culture gave C XI. Two 24-hr. blood-agar slants subcutaneously in rabbits. Lived.

In this group, Series C, there are eleven organisms. All of them were cocci occurring in pairs and chains. They were round, flattened, or appeared slightly lancet-shaped. Gram stain was always positive. Capsules could never be demonstrated by the methods employed. On blood-agar the growth was a dryish film composed of innumerable minute colonies which gave the surface a granular appearance; after some days' growth the blood was frequently hæmolysed. In serum there was no cloud but a granular sediment, and as a rule there was no acid produced. Rarely the fluid became neutral or gave litmus a faint reddish tinge. There was nothing to compare with the acid production seen in group B. Inulin-serum water was never acidified or clotted. The organisms rarely killed rabbits, and in the animals which died definite lesions were often missed. Once an arthritis developed.

Finally, we have Series A, the organisms obtained from the mouths of healthy individuals. In this series, as shown in the following tables, there are forty-two organisms. These cocci were distinguishable into two types, to be called Type I and Type II.

TABLE III.

Series A. Type I.

No. of Organism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
I.	Hospital resident.	11/2	Lanceolate diplococci, few chains. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	1 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 330 grams; death in 36 hrs. Extensive sero-fibrinous subcutaneous exudate; small, hard spleen. Cultures from heart's blood and subcutaneous exudate positive. The organism was passed through 18 rabbits; fibrinous subcutaneous exudate and fibrino-purulent peritonitis were the usual lesions; in several instances sero-fibrinous pericarditis and double pleurisy. Once extensive fibrino-purulent mediastinitis. Spleen usually hard and large, sometimes soft and small. Rabbit 19, wt. 1290 grams. 0.05 c.c. 24-hr. bouillon culture intraperitoneally; death in 33 days. Pneumococcus septicæmia.

TABLE III (*Continued*).

No. of Organism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
VII.	Laboratory janitor.	4/14	Lanceolate cocci in pairs. Gram positive.	Positive; blood-agar (Hiss).	9 days, acid, thickening; no firm clot.	Three 24-hr. blood-agar slants subcutaneously in rabbit, wt. 790 grams; death in 7 days. Extensive subcutaneous fibrinous exudate; ulceration of skin; spleen soft. Cultures from heart and subcutaneous tissues positive.
VIII.	Laboratory assistant.	11/26	Lanceolate cocci in pairs. Gram positive.	Positive; blood-agar (Hiss). Positive; serum (ordinary stain).	24 hrs., clot.	One 24-hr. blood-agar culture intraperitoneally in rabbits; lived. Small doses have no effect.
X.	Hospital resident.	11/29	Small lanceolate cocci in pairs and short chains. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	2 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 900 grams. Lived. 1 c.c. 24-hr. bouillon culture subcutaneously in mouse; death in 36 hours. Extensive sero-fibrinous exudate. Cultures from peritoneum and heart positive.
XIII.	Outdoor physician.	12/1	Lanceolate and round cocci in pairs and short chains. Gram positive.	Positive; serum (Hiss).	24 hrs., clot.	2 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 980 grams; death in 28 days. No local lesions; general septicæmia. Cultures from heart positive. 1 c.c. 24-hr. bouillon culture intraperitoneally in mouse; death in 12 hrs. Cultures from heart and peritoneum positive.
XIV.	Hospital resident.	12/6	Lanceolate and round cocci in pairs. Gram positive.	Positive; serum (Hiss).	24 hrs., clot.	1 c.c. 24-hr. bouillon culture subcutaneously in mouse; death in 60 hrs. Extensive sero-fibrinous exudate. Cultures from subcutaneous tissues and heart positive. 2 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 1250 grams. Lived.
XV.	Hospital resident.	12/6	Lanceolate diplococci, no chains. Gram positive.	Positive; serum (Hiss).	4 days, clot.	2 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 1190 grams; death in 36 hrs. Sero-sanguineous subcutaneous exudate, acute sero-purulent peritonitis. Culture from heart positive.
XVI.	Laboratory assistant. Same organisms in plates from sputum.	12/6	Round and lanceolate cocci in pairs and short chains. Gram positive.	Positive; serum (ordinary stains).	4 days, clot.	4 c.c. 24-hr. bouillon culture intraperitoneally in rabbit. Lived. Two 24-hr. blood-agar cultures intraperitoneally in rabbit; death in 6 days. Slight fibrinous exudate in subcutaneous tissues, fibrino-purulent peritonitis; large, soft spleen. Cultures from heart and peritoneum positive.

TABLE III (*Continued*).

No. of organism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
XVII.	Hospital resident. Same organism from saliva in plates.	12/13	Lanceolate cocci in pairs and short chains. Gram positive.	Positive; serum (Hiss).	3 days, clot.	4 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 1570 grams; death in 4 days. Extensive sero-fibrinous exudate in subcutaneous tissues; small, soft spleen. Culture from heart and subcutaneous tissues positive. Virulence gradually raised. In various rabbits there were produced sero-fibrinous and hæmorrhagic subcutaneous exudates, subcutaneous œdema, sero-purulent peritonitis, pleurisy, and pericarditis, extensive broncho-pneumonia, and hæmorrhagic septicæmia. After passage through 16 rabbits, 0.001 c.c. 24-hr. bouillon culture kills rabbit, wt. 480 grams, in 12 hours.
XVIII.	Laboratory assistant. Same organism from saliva in plates.	12/13	Lanceolate and round cocci in pairs and long chains. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	4 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 2240 grams; death in 24 hrs. Fairly extensive sero-fibrinous exudate in subcutaneous tissues. Spleen small and soft. Cultures negative. One 24-hr. blood-agar culture subcutaneously in rabbit, wt. 1240 grams; death in 7 days. Extensive subcutaneous congestion and fibrinous exudate; sero-purulent peritonitis. Cultures from heart and peritoneum positive.
XIX.	Workman; open air.	12/14	Lanceolate and round cocci in pairs. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	One 24-hr. blood-agar slant intraperitoneally in rabbit, wt. 1380 grams. Death in 35 days from secondary infection.
XX.	Workman; open air. Same organism from plates.	12/14	Lanceolate, round, and sometimes flattened cocci in pairs and chains. Gram positive.	Positive; serum (Hiss).	24 hrs., clot.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 1700 grams; death in 36 hrs. No local lesions; large, hard spleen. Cultures and cover-slips from heart positive.
XXI.	Physician; outdoor.	1/20	Lanceolate cocci in pairs, no chains. Gram positive.	Positive; serum (Hiss).	36 hrs., clot.	After passage through one rabbit, 1 c.c. 24-hr. bouillon culture intraperitoneally in rabbit, wt. 890 grams; death in 12 hrs. No local lesions. Culture from heart positive.

TABLE III (*Continued*).

No. of Organism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
XXII.	Hospital interne.	1/25	Lanceolate cocci in pairs. Gram positive.	Positive; serum (Hiss).	24 hrs., clot.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 680 grams; death in 36 hrs. Extensive subcutaneous fibrinous exudate. Small soft spleen. Cultures from subcutaneous tissues and heart's blood positive. 1 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 1200 grams; death in 11 days. Sloughing sore in abdominal wall. Cultures from heart, peritoneum, and subcutaneous tissues negative.
XXIII.	Laboratory janitor.	1/28	Lanceolate and round cocci in pairs and short chains.	Positive; serum (Hiss).	24 hrs., clot.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 1200 grams; death in 41 days. Secondary infection.
XXVII.	Physician; after sea voyage.	2/23	Lanceolate and round cocci in pairs and few short chains. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	5 c.c. saliva in rabbit, wt. 390 grams; death in 48 hrs. Extensive sero-fibrinous exudate in subcutaneous tissues. Cultures from heart and subcutaneous tissues positive.
XXVIII.	Laborer.	2/27	Lanceolate cocci in pairs, no chains. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 900 grams; death in 4 days. Moderate subcutaneous fibrinous exudate; spleen large and hard. Cultures from heart positive. 1 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 900 grams; death in 33 days. Much emaciation. Cultures from heart negative.
XXXIII.	Physician; acute coryza; nasal swab.	3/19	Lanceolate cocci in pairs and long chains Gram positive.	Positive; blood-agar (ordinary stains).	No clot, 10 days.	After passage through 5 rabbits, 0.5 c.c. 24-hr. bouillon culture intraperitoneally in rabbit, wt. 620 grams; death in 12 hrs. Sero-sanguineous peritonitis; large, hard spleen. Cultures from peritoneum and heart positive. Organism produces sero-fibrinous subcutaneous exudate, subcutaneous œdema, and fibrino-purulent peritonitis.
XXXVIII.	Physician.	4/6	Lanceolate and round cocci in pairs and short chains. Gram positive.	Positive; serum (Hiss).	48 hrs., clot.	After passage through 2 rabbits, 1 c.c. 24-hr. bouillon culture intraperitoneally in rabbit, wt. 350 grams; death in 5 days. Extensive fibrinous peritonitis; spleen enlarged and soft. Cultures from peritoneum and heart positive. In other animals subcutaneous sero-fibrinous exudates were produced.

In this group have been included all the organisms which resembled the cocci of Series B. There are nineteen which could not be differentiated by the methods employed from the organisms classed in this latter group. The morphology and the staining reactions were the same; capsules could always be demonstrated with ease by Hiss' method and the cocci stained by Gram's method. On blood-agar the same watery type of growth was observed, and in the stock serum a diffuse cloud and large quantities of acid were produced. Inulin was fermented and inulin-serum water acidified and clotted in all but one instance (A XXXIII). This organism is comparable to B X. It was fairly virulent for animals, producing extensive fibrinous exudates in the subcutaneous tissues, fibrino-purulent peritonitis, and the hard, swollen spleen infiltrated with fibrin.

The acid production in inulin was sometimes slower and less marked than with the B series. Indeed in the whole group of organisms which fermented inulin quite a variation existed in the amount of acid produced as could be shown by tritration with $\frac{1}{10}$ N. sodium hydroxide.

The organisms belonging to Type I of Series A were, as a rule, much less virulent for rabbits than those of Series B. Many were practically non-pathogenic, and none in their original cultures showed any high grade of virulence. The virulence could, however, be exalted by successive passages through animals. For instance, with A XVII, after passage through sixteen rabbits, 0.001 c.c. of a twenty-four-hour bouillon culture killed medium-sized rabbits. The elevation of virulence was, however, in some cases very difficult, as in A I, where it required passages through nineteen rabbits to produce a culture capable of killing rabbits in doses of 0.05 c.c. of a twenty-four-hour bouillon culture. The lesions in the animals which died were in every respect like those produced by the cocci of Series B. During the elevation of virulence a certain sequence of lesions was apt to occur. First, extensive fibrinous exudates in the subcutaneous tissues or peritoneum, if the inoculation was made intraperitoneally; later, fibrinous or fibrino-purulent exudates in the pleuræ or pericardium, and finally, general septicæmia without local lesions. Two

animals lived for several weeks at the end of which time they died of a general septicæmia, cultures and cover-slips from the heart's blood at autopsy showing enormous numbers of pneumococci.

The organisms of Type II differed in many respects from the above description.

TABLE IV.

Series A. Type II.

No. of Organism	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
II.	Outdoor physician.	11/2	Lanceolate and round diplococci. Gram positive.	Blood-agar; suggestive	No clot, 10 days.	1 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 345 grams. Killed accidentally after 5 days. Cultures from blood positive. No subcutaneous exudate.
IV.	Laboratory assistant.	11/23	Small round cocci in pairs and clusters. Gram positive.	Negative; serum (Hiss).	No clot, 10 days.	1 c.c. 24-hr. bouillon culture subcutaneously in mouse. Animal lived. 2 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 2000 grams. Lived.
VI.	Hospital resident.	11/26	Small round cocci in pairs and long chains. Gram positive.	Negative; serum (Hiss).	No clot, 8 days.	2 c.c. 24-hr. bouillon culture subcutaneously in rabbit, wt. 1400 grams; lived. One rabbit during immunization died 14 days after intraperitoneal injection of 3 c.c. 24-hr. serum bouillon culture. General septicæmia. Cultures from heart and peritoneum positive. No other animals could be killed, though large doses were used both subcutaneously and intraperitoneally.
XIII.	Outdoor physician.	12/1	Small, round, and flattened cocci in pairs and long chains. Gram positive.	Negative; serum (Hiss).	No clot, 6 days.	2 c.c. 24-hr. bouillon culture intraperitoneally in mouse; death in 6 days. No local lesions; cultures from peritoneum and heart positive. One 24-hr. blood-agar culture intraperitoneally in rabbit, wt. 1400 grams. Lived.
XXV.	Gatekeeper.	2/14	Long chains of round and flattened cocci. Gram positive.	Negative; serum (Hiss).	No clot, 10 days.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 1190 grams; death in 14 days. Abscess in subcutaneous tissues. Cultures negative.

TABLE IV (*Continued*).

No. of Organism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
XXVI.	Workman.	2/17	Small, round, and lanceolate cocci, often in pairs and short chains. Gram positive.	Negative; serum (Hiss).	No clot, 7 days.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 650 grams; death in 3 days. Small pea-sized localized abscess at point of inoculation. Cultures negative.
XIX.	Elevator boy.	3/14	Small, round, and lanceolate cocci in pairs, groups, and chains. Gram positive.	Negative; serum (Hiss).	No clot, 8 days.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 680 grams; death in 41 days. No local lesions. Cultures negative.
XXX.	Book-binder.	3/6	Lanceolate, round, and oval cocci in pairs. Gram positive.	Positive; serum (Hiss).	No clot, 8 days.	One 24-hr. blood-agar slant, subcutaneously in rabbit, wt. 430 grams; death in 27 days. No local lesions. Cultures negative.
XXV.	Laborer.	3/29	Round and lanceolate cocci in pairs and chains and groups. Gram positive.	Negative; serum (Hiss).	No clot, 7 days.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 909 grams; death in 7 days from rabbit septicæmia.
XXVI.	Pressroom worker.	3/29	Round and lanceolate cocci in pairs and chains. Gram positive.	Negative; serum (Hiss).	No clot, 7 days.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 950 grams; death in 14 days. Localized abscess filled with white, grumous material at point of inoculation. Cultures from abscess and organs negative.
XXVII.	Hospital resident.	4/1	Lanceolate cocci in pairs and chains. Gram positive.	A few organisms have capsules; serum (Hiss).	No clot, 8 days.	Three 24-hr. blood-agar slant intraperitoneally in rabbit. Lived.
XVIII.	Physician.	4/6	Small, round, and lanceolate cocci in pairs and chains. Gram positive.	Negative; serum (Hiss).	No clot, 8 days.	Three 24-hr. blood-agar slants intraperitoneally in small rabbit. Lived.

TABLE IV (*Continued*).

No. of Organism.	Source.	Date.	Morphology.	Capsules.	Inulin-Serum Water.	Pathogenicity.
XXXIX.	Physician.	4/6	Small, round, and slightly lanceolate-shaped cocci in pairs and long chains.	Negative; serum (Hiss).	No clot, 9 days.	One 24-hr. blood-agar slant subcutaneously in rabbit, wt. 340 grams; death in 1½ days. No local lesions. Cultures negative.
XL.	Laboratory assistant, same as A I.	4/24	Round and lanceolate cocci singly, in pairs, and groups. Gram positive.	Negative; serum (Hiss).	No clot, 8 days.	Three 24-hr. blood-agar slants intraperitoneally in rabbit, wt. 300 grams; death in 12 hrs. Fibrino-purulent peritonitis; spleen large and soft. Cultures from heart and peritoneum positive.
XLII.	Laboratory assistant.	4/19	Round and lanceolate cocci in pairs and short chains. Gram positive.	Negative; serum (Hiss).	No clot, 11 days.	Two 24-hr. blood-agar slants intraperitoneally in small rabbit. Living 12 days after.
XLIII.	Physician; same as XVII A. Mouse lived. Cultures in plates from saliva.	4/26	Small, round, and flattened cocci in pairs and chains.	Negative; serum (Hiss).	No clot, 10 days.	2 c.c. saliva in white mouse. Lived.

Of these there were sixteen, and though not conforming in every particular to the class of cocci grouped in Series C, they came much closer to them than to those in Series B.

They appeared usually as diplococci, often having a lancet-shape, but with a tendency to form round and flattened forms arranged in chains. They stained positively by Gram's method. Capsules could not be demonstrated except in rare instances and then only with the special stains of Hiss when they were quite indefinite. The growth on blood-agar was dryish and formed a fine granular film. So characteristic was this appearance that it could frequently be predicted from the blood-agar growth whether the organisms belonged to Type I or Type II, and in two instances a mixed culture was detected by this means. No

acid and no clot was ever produced in inulin-serum water. In serum there was no cloud, but the growth settled to the bottom as a granular sediment. Traces of acid were occasionally produced, and in this respect the organisms differed slightly from those belonging to Series C.

As a rule, these organisms showed a very slight grade of virulence. In large doses they did sometimes kill rabbits, but the organism could rarely be cultivated from the organs at autopsy. The lesions consisted of small subcutaneous abscesses and ulcers of the skin. None of the lesions described for Type I and Series B could be produced by any of the organisms of this group.

From the saliva of two different individuals organisms belonging to both Type I and Type II were isolated and separated satisfactorily. In both instances the cocci were recovered from the tissues of a mouse inoculated with 2 c.c. of saliva.

By treating these organisms with human blood, according to the method of Wright and Douglas for the demonstration of the opsonic power of the serum, it was discovered that the leucocytes of normal individuals ingested the cocci of Series C and Series A, Type II, as far as they were tested, but did not take up the organisms belonging to Series B and Series A, Type I. Unfortunately it was only possible to make tests with a few members of each group, so that these results are rather suggestive than conclusive. By reason of the above differences it was thought justifiable to separate this large group of cocci of Series A into the two types just described.

Altogether forty specimens of saliva were examined. The method was to inoculate 2 c.c. of saliva subcutaneously into a white mouse, and at autopsy to make cultures from the subcutaneous tissues, peritoneum, and heart's blood. In certain instances plate cultures were also made from the saliva. In four instances we succeeded in obtaining the same organism from plates and from the inoculated animal.

From the 40 cases, organisms were isolated 33 times, or in 82.5 %. In 19 cases, or in 47.5 %, Type I was obtained; in 16 cases, or in 40 %, Type II. In two instances both organisms were isolated.

In the table below, the organisms are tabulated according to the occupation of the individual from whom the saliva was taken.

TABLE V.

Occupation.	Total No. of specimens of saliva examined.	Positive.	Negative.	Percentage of positive results.	Type I.	Per cent.	Type II.	Per cent.
Hospital interne.....	11	8	3	72.7	6	54.5	2	18.2
Laboratory assistants and janitors.....	9	8	1	88.8	5	55.5	3	33.3
Physicians living in town and country. .	12	10	2	83.3	5	41.6	5	41.6
Laborers working out-of-doors.....	7	6	1	85.7	3	42.8	3	42.8
Laborers; indoor occupation.....	3	3	—	100	—	—	3	100
	42	35	7	83.3 %	19	45.2 %	16	38.1 %

Though the number of cases is small, still the relative percentages of the two types of organisms and of the total number of positive results remain fairly constant.

In the table below the organisms are arranged according to the month of the year in which the saliva was examined.

TABLE VI.

Month.	Total No. of specimens of saliva examined.	Positive.	Negative.	Per cent Positive.	Type I.	Per cent.	Type II.	Per cent.
November.....	9	6	3	66.6	3	33.3	3	33.3
December..	9	9	0	100	8	88.8	1	11.2
January.....	3	3	0	100	3	100	0	0
February.....	5	4	1	80	2	40	2	40
March..	7	5	2	71.4	1	14.2	4	57.2
April..	9	8	1	88.8	2	22.2	6	66.6
	42	35	7	83.3 %	19	45.2 %	16	38.1 %

In this table the variation in the percentages of the two types of organism is very great, and though the number of examinations is too small to permit conclusions to be drawn, still the results are suggestive.

In November, the percentage of the true pneumococcus type is not very large; in December and January it increases enormously, to fall again gradually to a low level in March and April.

In one instance (A I, Type I), a fairly virulent member of Type I was recovered from the saliva in November, while in April (A XL) only organisms belonging to Type II were obtained from the saliva of the same individual. Again, in another instance, in December, a very virulent organism (A XVII, Type I) was recovered from the saliva both by plating and animal inoculation, while in April the saliva from the same person produced no effect in mice, and in plates only organisms belonging to Type II (A XLIII) could be obtained.

These experiments suggest that the pneumococcus is not to be found constantly in the mouths of 40 % or 50 % of healthy individuals, as one might at first suppose from the tabulated results. The technique which we used was the same throughout our work, so that the failure to obtain a large percentage of pneumococci in the spring months could scarcely depend upon this factor. Of course the series of cases is far too small to enable one to draw conclusions, but the results suggest that during the winter months the pneumococcus has a wide distribution, and that at this time a large percentage of healthy individuals harbor virulent pneumococci in their buccal cavity. These months precede those in which pneumonia is most prevalent. It is almost certain that some persons always have virulent pneumococci in their saliva.

In conclusion we should like to express our thanks to Dr. Alfred Stengel, Dr. C. Y. White, and Dr. Nisbit, who placed the Pepper Clinical Laboratory at our disposal for the animal investigations, and who facilitated this part of the work by many kindnesses.

A STUDY OF PNEUMOCOCCI AND ALLIED ORGANISMS IN HUMAN MOUTHS AND LUNGS AFTER DEATH.

By CHARLES NORRIS, M.D., and ALWIN M. PAPPENHEIMER, M.D.[1]

(*From the Laboratories of Bellevue and Allied Hospitals, New York City.*)

Our studies have been confined especially to a determination of the prevalence of the pneumococcus in normal lungs, and in lungs which presented various lesions. At the same time, we have tried to determine, by experimental methods, how justly one may draw inferences with regard to the flora of the living lung from cultural findings after death.

Numerous investigations have shown that the pneumococcus is more or less constantly present in a variety of pulmonary lesions, especially in lobar pneumonia. The pneumococcus has been isolated from a considerable proportion of apparently healthy lungs, both human and animal, that have been examined after death. A similar micro-organism, or one closely related to it, has been found in the mouths of many healthy human beings.

The question of the presence of bacteria in normal lungs has long been an urgent one, because of its obvious bearing upon the determination of the occurrence of the infectious diseases of this organ. If organisms are present in all lungs, it may be supposed that the pneumococcus will be more frequently found in the lungs of patients exposed for some time to a hospital atmosphere. This point, too, we have tried to decide, and for this reason we have classified our cases into the following three groups, viz:

I. Patients dying outside of hospital.

II. Patients dying in hospital within twenty-four hours of admission.

[1] Aided by a grant from the Commission for the Investigation of Acute Respiratory Diseases of the Department of Health of New York City.

III. Patients dying in hospital twenty-four hours or longer after admission.

This report embodies the results of the bacterial examination of a series of forty-two (42) human lungs. The cases were taken at random, so as to comprise a variety of lesions. There were included cases which died in the wards of Bellevue Hospital, and a lesser number of coroner's cases, which died either outside the hospital or in the hospital within twenty-four hours after admission.

These lungs were selected from subjects examined within twenty-four hours after death. The bodies were removed from the wards, usually within an hour after death, and kept in cold storage until examination.

METHODS EMPLOYED IN THE STUDY OF THE BACTERIA IN THE MOUTH AND LUNGS.

Isolation.—The isolation of the organisms studied was carried out in the following manner: The lung to be cultivated was clamped before removal, by a large hysterectomy clamp, which firmly occluded the large vessels and bronchus. This was done to prevent, as far as possible, the aspiration of fluid or mucus from the trachea, during the manipulation of removing the lungs. The surface of the lung was thoroughly seared with a large knife over the posterior portion of the lower lobe. The juice was obtained in the usual manner, by stabbing the sterile area with a heated Nuttall spear, and was then thinly streaked on glycerine-agar plates. The fluid suspension of material from the lungs for the inoculation of the mice was obtained as follows: With sterile forceps and scissors, a large piece of lung was excised from the seared area and cut into small pieces in a sterile Petri dish; in cases where the juice expressed was insufficient a little broth was added. From 0.5 to 1.0 c.c. was inoculated into the skin of the back. In the majority of cases white mice were used, but, owing to the difficulty of obtaining a sufficient number, we were compelled (in a few instances which are noted) to use colored or spotted mice.

From the lung plates, after from twenty-four to forty-eight hours, sub-cultures were made on Loeffler's blood-serum from pneumococcus- or streptococcus-like colonies; usually as many as half a dozen were transplanted.

From the heart's blood of mice dying after inoculation, streak plates were made on glycerine-agar, and the colonies which had developed after from twenty-four to forty-eight hours were sub-cultured on Loeffler's blood-serum. In case the colonies varied in appearance, several sub-cultures were always made from each type.

For the isolation of the pneumococcus from saliva we have depended almost wholly upon the subcutaneous inoculation of mice with a small amount of mucus from the mouth. This was obtained at autopsy, on a cotton swab, which was then shaken in broth and the suspension injected. The subsequent proceedings were identical with those above described for the lungs. Glycerine-agar plates were made in a few instances, but, on account of the difficulty encountered in obtaining satisfactory plates, this was not done as a routine.

Morphology.—The morphology of the bacterial flora of the lung was studied in cover-slip preparations and in sections of lung tissue. As a routine stain for the demonstration of capsules, the two staining methods as described by Hiss [2] were employed; in smears from the lung tissue, a Gram preparation was also made. Sections were stained for bacteria by the Gram-Weigert method.

Cover-slip preparations from the heart's blood of mice, from typical colonies on plates, from the primary sub-cultures on Loeffler's medium, were examined as a routine, and in many instances from the transplantations on different media.

Determination of Cultural Characters.—For the study of cultural characteristics, transplants were made from the primary Loeffler sub-cultures upon the following media: broth, gelatine, glycerine-agar slants, litmus-milk, Hiss' 1 % inulin-serum water (1:3). In addition, the fermentative activities of a certain number of what we may for the present call the different

[2] *Journal of Experimental Medicine*, 1905, vi, 317.

strains of the pneumococcus, as well as the streptococcus, were studied on a series of sugar media containing dextrose, lactose, maltose, saccharose, mannit, or glycogen, and on dextrin and soluble starch.[3]

Determination of Virulence.—This was limited to the inoculation of mice with half or whole cultures, grown for twenty-four hours on glycerine-agar slants. Unfortunately, we were not in a position to determine accurately the virulence of the various strains, owing to the insufficient supply of mice at our disposal. Because of this scarcity we rarely were able to ascertain the virulence of the organism in fresh isolations.

DESCRIPTION AND CLASSIFICATION OF THE PNEUMOCOCCI, STREPTOCOCCI, AND ALLIED FORMS, FOUND IN THE LUNGS AND MOUTHS.

In consonance with the opinions of more recent observers, we believe that the older criteria relied upon for the differentiation of pneumococci and streptococci are insufficient. Even the fermentative activities upon which so much stress has recently been laid have not been, in our hands, an infallible guide, especially with reference to the identification and classification of the intermediate types of these two closely related cocci, of which many have been encountered.

We must likewise take exception to the older and perhaps still widely prevalent view, that streptococci, unlike the pneumococci, may not produce a bacteriæmia in mice, as in many cases we were able to isolate streptococci in cultures from the heart's blood of mice taken immediately after death.

In order to describe the bacteria we have encountered during the course of this work we have been forced, somewhat against our will, to adopt an arbitrary classification founded mainly upon

[3] The sugars were obtained from Merck. The inulin employed by us in the earlier determinations was obtained from Merck. This inulin was found highly unsatisfactory, from the presence of resistant spores. All our later work was done with Kiliani's inulin, "Eimer and Amend." Many of our earlier cultures were tested again upon this same inulin. Kahlbaum's soluble starch was used for the starch-serum water, in the strength of two per cent.

the variations in morphological and physiological characters. The most important and constant of these are the presence or absence of capsules, and the fermentation or non-fermentation of inulin. Our grouping of these cocci is as follows:

GROUP I.

Typical pneumococci.—By this, we designate those diplococci which ferment inulin with acid formation, and coagulation of the serum; which possess readily stained capsules, not only in the blood of mice, but on various media; which give a diffuse cloudiness in broth, and grow as fine, colorless, delicate, translucent colonies on glycerine agar, and which are virulent to mice. We have met with a number of organisms which, although fermenting inulin with slight acid production, do not coagulate the serum. This, we consider, indicates, in general, feeble growth.

GROUP II.

Streptococcus mucosus.—By this term, we refer to a series of organisms which differ from the typical pneumococcus in their abundant, moist, or mucous growth upon various solid media and in the more constant production of chains upon media, but which resemble the pneumococcus in possessing a constantly demonstrable capsule, in their fermentative activity, and in their virulence to mice.

In conformity with the observations of other observers, we have found that the capsules of these diplococci are more easily demonstrable than those of the typical pneumococcus, and that there are certain variations in the structure of the capsules, which we have not observed in those of the pneumococcus. We have found that the cocci of this group grow more constantly and abundantly at room temperature in gelatin.

We have not as yet been able to decide definitely, whether the group constitutes a distinct variety of the pneumococcus, or whether the mucous character of the growth and chain formation is dependent upon variations in the media. Thus we have found that on dry Loeffler's serum tubes the growth of these organisms may be dry, whereas on wet tubes it tends to be mucous-like in character. We have also seen organisms resembling the pneumococcus, after passage through mice, assume a viscid character of growth. In fact, there seem to be numerous gradations between these two types in this respect.

GROUP III.

Diplococci resembling pneumococci which possess no demonstrable capsule.—No capsules have been demonstrated in these diplococci, either in the heart-blood of mice, or on culture media after repeated examination. They produce acid in inulin, but do not coagulate the serum. In all other respects, they resemble pneumococci.

GROUP IV.

Diplococci resembling streptococci which possess no demonstrable capsule.—No capsules have been demonstrated in these diplococci, either in the heart-blood of mice, or on culture media. They are active inulin fermenters. These cocci, although fermenting inulin with acid production, have greater points of similarity to the streptococcus than to the pneumococcus. They grow abundantly in artificial media, produce clouding, or flocculi and granules in broth, grow readily at room temperature, and show a marked tendency to chain formation.

GROUP V.

Typical streptococci.—These have no capsules, do not ferment inulin with acid production, and grow readily at room temperature. They produce clouding in broth, or form granules or flocculi adherent to the sides of the tubes.

SOURCES OF ERROR IN TECHNIQUE.

It is obvious that there are many possibilities of error in the isolation of pneumococcus-like organisms from the lungs by methods above detailed. On account of the similarity in the colonies on glycerine-agar, and even on ascitic-agar, there is always difficulty in distinguishing on inspection pneumococcus from short-chained streptococcus colonies, and in consequence the pneumococcus, though present in small numbers, may escape detection, for it is obviously not practical to sub-culture more than a limited number of suspicious colonies.

Overgrown plates are another but not a frequent source of trouble. The organisms responsible for this are not only those which are introduced through natural errors in technique, but also those which we have found more or less constantly present in the lungs cultivated after death, as verified by a thorough examination of cover-slip preparations from the lung tissue.

Again, another source of error lies in the natural insusceptibility of white mice, and even more so of certain strains of colored mice, to infection by pneumococci. Thus it has been found by us that colored mice are more resistant to infection than white mice when inoculated with similar doses. Moreover, it has been found that in some of our cases even a white mouse does not succumb to the inoculation of a comparatively large amount of expressed lung juice, although cover-slip preparations

of the lungs have shown typical capsulated diplococci, positive to Gram. For this reason, so far as it has been possible, we have inoculated two mice from each case.

Still another though less frequent and serious difficulty is that encountered in the isolation of pneumococci in streak agar plates from the heart blood of mice dying of mixed infection. In a certain number of cases we have found Bacillus mucosus capsulatus to be the predominating organism; in other cases, non-capsulated cocci which we have not as yet identified. In a certain number of cases also, though pneumococcus-like organisms were present in smears from the lung, the mice inoculated died from streptococcus, Bacillus mucosus capsulatus, or other infections. On the other hand, we have encountered capsulated cocci in the heart's blood of mice, as well as upon our lung plates, which failed to grow on transplantation upon glycerine-agar or Loeffler's blood-serum.

With this brief introduction, we can now present more readily the result of our work largely in tabulated form. The tables which follow must be briefly described.

The first column of the tables, headed "Case Number," indicates the number of the case in our series. The next column, the "Accession Number," refers to the entry number of each case in the autopsy accession book of the hospital. The Roman numerals I, II, III, IV, and V are those we have used to designate the five groups into which we have divided the pneumococci, streptococci, and the cocci allied to them.[4] The addition sign (+) or the zero sign (o) opposite each case indicates that a coccus of this group has or has not been isolated.

[4] I.—Pneumococcus.
II.—Streptococcus mucosus.
III.—Diplococci without capsules, which ferment inulin only with acid production.
IV.—Diplococci without capsules, which are active inulin fermenters, and which closely resemble streptococci.
V.—Streptococci.

PNEUMOCOCCI, STREPTOCOCCI, AND ALLIED MICRO-ORGANISMS IN MOUTHS AFTER DEATH.

In fourteen cases the mouths were cultivated by the methods sufficiently described above. We append the analysis of our findings:

TABLE I.

ANALYSIS OF PNEUMOCOCCI, STREPTOCOCCI, AND ALLIED MICRO-ORGANISMS, ISOLATED FROM THE MOUTH AFTER DEATH.

	Case No.	Accession No.	Group I.	Group II.	Group III.	Group IV.	Group V.	Unidentified.
	1.	82	+	o	o	o	o	(?)
	2.	98	o	o	o	o	+	
	3.	105	o	o	o	o	+	
	4.	110	+	o	o	o	o	
	5.	153	o	o	o	+	o	
	6.	164	o	o	o	o	+	
	7.	165	+	o	o	o	o	
	8.	166	+	o	o	o	o	
	9.	167	o	o	o	o	o	+
	10.	170	o	o	o	o	+	
	11.	172	o	o	o	o	o	+
	12.	191	+	o	o	o	+	
	13.	199	o	o	+	o	o	
	14.	233	o	o	o	o	o	
Total	14 cases		5	o	1	1	5	2

The pneumococcus, as shown by Table I, was isolated five (5) times, the streptococcus five (5) times, and intermediate organisms twice. Of these twelve cases, all but three were in the hospital over twenty-four hours. Two died with lobar pneumonia; in one of these, the mouse injected with mouth secretion died of streptococcus infection, and this organism, in pure culture, produced a bacteriæmia fatal within twenty-four hours in a mouse inoculated with three-fourths of a glycerine-agar growth twenty-four hours old.

The virulence of the cocci isolated was tested in eight (8) cases. Three (3) of these were pneumococci, four (4) were streptococci, and one (1) Group IV. One of the streptococcus cultures proved to be non-virulent. All the others killed the

mice in from eighteen to forty-eight hours, partial or whole cultures being inoculated.

All the mice inoculated with mouth secretion died, in most cases, within twenty-four hours. Mice inoculated with the secretion from two cases died with infections other than pneumococcus or streptococcus. From a third case, one of the mice inoculated died after four (4) days, no organisms being recovered in smears or by culture; the second mouse of the same case remained alive.

These few observations seem to show that the streptococci isolated from the mouth were quite as virulent as the pneumococci. The pneumococci obtained from two (2) cases not dying of lobar pneumonia were found to be quite as virulent as those obtained from pneumonic lungs post-mortem. Further conclusions, however, as to the general virulence of the pneumococci obtained from the mouth do not seem to be justified.

We have made no attempts to determine the frequency of pneumococci in the mouths of ward patients or attendants on account of an insufficient supply of mice.

PNEUMOCOCCI, STREPTOCOCCI, AND ALLIED BACTERIA ISOLATED FROM THE LUNGS AFTER DEATH.

Forty-two (42) cases were examined. The analysis of our findings follows in tabulated form (Table II).

Table III shows the number of cases in which the various groups of cocci have been found, either alone or in association with the other groups.

We are now in a position to analyze the facts presented above, in Table IV, which requires a brief explanation. The cases cultivated in this research have been divided into three classes, according to whether the lungs were normal in the gross or presented the lesions of lobar pneumonia, or were the seat of a variety of other lesions. Each of these classes has again been divided into three classes, viz., those cases dying outside the hospital, those dying in the hospital within twenty-four hours of admission, and those dying twenty-four hours or more after admission.

TABLE II.

ANALYSIS OF THE DIPLOCOCCI ISOLATED FROM THE LUNGS.[5]

Case No.	Accession No.	Group I.	Group II.	Group III.	Group IV.	Group V.	Unidentified.
1.	43	o	o	o	o	o	
2.	45	o	o	o	o	+	
3.	46	o	o	o	o	+	
4.	49	+(?)[6]	o	o	+	+	
5.	52	o	o	o	+	o	
6.	54	o	o	o	+	o	
7.	63	+	o	+	+	o	
8.	68	+(?)	o	o	o	o	
9.	70	+(?)	o	o	o	o	
10.	71	o	+	o	o	o	
11.	82	+	o	+	o	o	
12.	98	o	o	o	o	+	
13.	102	o	o	o	o	+	
14.	105	+(?)	o	o	o	+	
15.	110	+	o	o	o	o	
16.	113	o	o	o	o	+	
17.	114	+(?)	o	o	o	+	
18.	123	o	o	o	o	o	+
19.	140	+	+	+	o	o	
20.	147	o	o	o	o	o	
21.	149	o	o	o	+	o	
22.	153	o	o	+	o	o	
23.	160	o	+	o	o	o	
24.	164	o	o	o	o	+	
25.	165	+	o	o	o	+	
26.	166	+	o	o	o	+	
27.	167	o	o	o	o	+	
28.	170	+	o	o	o	+	
29.	172	+	o	o	o	o	
30.	175	o	o	o	o	+	
31.	191	+	o	o	o	o	
32.	199	o	o	+	o	+	
33.	C I	+	o	o	+	o	
34.	C II	o	o	o	+	+	
35.	C III	+	o	o	o	o	
36.	C IV	+	o	o	o	o	
37.	C V	o	o	o	o	+	
38.	C VI	o	o	o	o	+	
39.	C VII	+	o	o	o	o	
40.	233	+	o	+	o	+	
41.	237	+	o	o	o	o	
42.	258	+	o	o	o	o	

[5] See explanation of Table I, p. 456.

[6] The cocci of Group I which are marked (?) were not absolutely identified as pneumococci, but they in all probability belong to the group, and they have therefore been included.

TABLE III.

ANALYSIS OF THE DIPLOCOCCI ISOLATED FROM LUNGS.

Group of Organisms.[7]	No. of Times Found.	Accession No. of Cases.
I alone,	10	68, 70, 110, 172, 191, C III, C IV, C VII, 237, 258.
II "	2	71, 160.
III "	1	153.
IV "	3	52, 54, 149.
V "	10	45, 46, 98, 102, 113, 164, 167, 175, C V, C VI.
I + II	0	
I + III	1	82.
I + IV	1	C I.
I + V	5	105, 114, 165, 166, 170.
II + III	0	
II + IV	0	
III + V	0	
III + V	1	199.
IV + V	1	C II.
I + III + IV	1	63.
I + II + III	1	140,
I + IV + V	1	49.
I + III + V	1	233.
Unidentified.	2	123, 147.

The data presented in Table IV may be thus briefly reviewed:

I.—NORMAL LUNGS.

a. Two cases dying outside of hospital.—In one, the pneumococcus was isolated; in the other, only a streptococcus.

b. Four cases dying within twenty-four hours of admission.—In these (63, 68, 153, 223) the pneumococcus was isolated three times; one of these cultures (68), however, may be considered questionable.

It is interesting to note that likewise in three of the cases organisms of Group III (non-capsulated inulin fermenter) were found, and in two of the cases streptococci (Group V).

c. Eight cases, dying twenty-four hours or more after admission.—The pneumococcus was obtained in two cases: in 166, where it was associated with streptococcus (Group V); and in 258, where it occurred alone. In two cases (71 and 160), Streptococcus mucosus (Group II); in one case (149), Group IV; in four cases (98, 166, 167, C V.), streptococci (Group V).

The pneumococcus, if 68 be called a true pneumococcus, was thus isolated five times. It is, perhaps, noteworthy, that pneumococcus was isolated from the cases dying outside, and those less than twenty-four hours in hospital, in four out of six cases, 66 %, whereas it was isolated in only two out of eight cases which had been in the hospital over twenty-four hours, 24 %.

[7] See explanation of Table I.

TABLE IV.

ANALYSIS OF THE CASES, ACCORDING TO THE GROUPS OF DIPLOCOCCI, AND THE LESIONS FOUND IN THE LUNGS AT AUTOPSY AND ALSO ACCORDING TO THE LENGTH OF TIME OF THE CASES IN THE HOSPITAL.

	Normal.						Lobar Pneumonia.						Other Pulmonary Lesions.						Total.					
	No. of Cases.	Group I.	Group II.	Group III.	Group IV.	Group V.	No. of Cases.	Group I.	Group II.	Group III.	Group IV.	Group V.	No. of Cases.	Group I.	Group II.	Group III.	Group IV.	Group V.	No. of Cases.	Group I.	Group II.	Group III.	Group IV.	Group V.
Not in Hospital.....	2	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	1
In Hospital 24 hours or less.	4	3	0	3	1	1	3	1	1	2	0	1	2	2	0	0	1	0	9	6	1	5	2	2
In Hospital over 24 hours..	8	2	2	0	1	4	9	5	0	0	0	6	13	6	0	1	4	6	30	13	2	1	5	16
Total.....	14	6	2	3	2	6	13	7	1	2	0	7	15	8	0	1	5	6	42	21	3	6	7	19

SUMMARY OF TABLE IV.

Total number of cases cultured..... 42
Micro-organisms obtained in..... 41
Group I..... 21 (5 doubtful).
" II..... 3
" III..... 6
" IV..... 7
" V..... 19
Unidentified..... 2

II.—LOBAR PNEUMONIA.

Thirteen (13) cases, one dying outside the hospital, three (3) within twenty-four hours of admission, and nine (9) after a residence in hospital of twenty-four hours or over. The pneumococcus was isolated from seven of these cases; this includes one non-inulin fermenter (114), which, however, exhibited a capsule after the second passage through mouse, the culture having been taken from the pneumonic lobe.

Cultures were made in seven of the thirteen cases from the consolidated lobe; in two, from the non-consolidated. Of the remaining four (4) cases, which showed organization of the exudate (so-called organizing pneumonia), cultures were made from the involved lobe three (3) times; from the uninvolved lobe, once.

From the seven lobes showing a typical pneumonia with exudate, in three cases (140, C VII, 237) typical pneumococcus (Group I) was obtained; once (140) associated with Streptococcus mucosus (Group II); in two cases (105, the organism growing poorly, and 114, above mentioned) two typical pneumococci were isolated, associated with streptococci (Group V); in another case (199) Group III, and also a streptococcus, Group V; in another (113) Group V was isolated, no other organisms being found in this case.

Of two cases cultivated from the uninvolved lobes, one (110) gave a typical pneumococcus; the other (147) showed B. mucosus capsulatus in pure culture from the lungs in plates, and from the mouse in plates, no pneumococci having been found in the cover-slip preparations made from the heart's blood of mouse.

In three cases where the lobes with organizing pneumonia were cultured, in one (43) no bacteria were found in plates, the single mouse inoculated remaining alive; in two cases (46 and 102) streptococcus (Group V) alone was isolated.

In one case of organizing pneumonia (165), in which the involved lobe was cultured, a pneumococcus (Group I) and a streptococcus (Group V) were isolated.

III.—LESIONS OTHER THAN LOBAR PNEUMONIA.

Of the fifteen remaining cases, none died outside the hospital; two were in hospital less than twenty-four hours; in one of these (C I) pneumococcus and an organism of Group IV were found; in the other (C III) pneumococcus alone was found.

Of the thirteen cases over twenty-four hours in hospital, the pneumococcus was found in four cases; in two cases (49 and 70) organisms, probably pneumococci (Group I), were obtained. In Case 49, organisms of Groups IV and V were also isolated; in 70, no other organisms were found. In one case (123) an unidentified non-capsulated diplococcus, which did not grow on transplantation from the lung plates, was obtained; the mouse inoculated with lung tissue remained alive.

From the tabulated summary of Table IV we see that the total number of cases cultured was forty-two, and that in all but two

(2) cases micro-organisms were obtained. From these cases, pneumococci and streptococci were obtained in practically similar percentages—that is, in 50 %. These results, as far as the pneumococcus is concerned, agree with those obtained by Beco, 50 % pneumococci and 45 % streptococci in human lungs.[8] Boni,[9] in lungs of pigs, has also obtained similar percentages. Dürck,[10] in pneumonic lungs of children, obtained pneumococcus in cases of lobar pneumonia, some of which were organizing or unresolved pneumonias, in seven out of thirteen, 52 %, streptococci being found in the same proportion.

Discussion of the Significance of the Data above Summarized.—A consideration of these facts suggests a number of interesting questions: Are the pneumococci present in the exudate of lobar pneumonia similar to the pneumococci which have been found in the mouths of normal persons, presumably as harmless inhabitants? If so, they reach the lungs either through the lymphatics or the vascular channels at some time during life, or by inhalation, lodging there as harmless saprophytes until some as yet unknown change in their host causes them to acquire increased virulence. A second possibility is that pneumococci of greater virulence than those found in the mouth are inhaled with the air current during life, determining the onset of the disease. However, it is still an open question whether pneumococci are present in the lungs of normal persons during life. May not their presence in normal lungs, as found by post-mortem examinations, be explained by a terminal septicæmia, such as has been established for the streptococcus and other micro-organisms, or do the pneumococci of the mouth gain access to the air vesicles with the saliva, either aspirated during the death agony or, later, by gravitation consequent upon the manipulation of the body after death, in its transportation from the wards to the morgue?

These problems complicate the interpretation of the various factors concerned in the production of lobar pneumonia. With

[8] Beco, *Archiv. d. la medecine expérimentale*, 1889, xi, 317.
[9] Boni, *Deutsch. Arch. f. klin. Med.*, 1901, Bd. 69.
[10] Dürck, *Deutsch. Arch. f. klin. Med.*, 1897, Bd. 58.

the more theoretical aspects of the question, such as the disturbance in balance between the host and the inciting agent of the disease, we have not attempted to cope, except in so far as we have roughly determined the virulence for mice of the organisms isolated in our mixed series of cases.

The statistics obtained by the investigators mentioned above and by ourselves might lead one to the inference that bacteria of the pneumococcus or streptococcus group exist during life in practically all lungs, whether normal or diseased. But, as we have already suggested, grave theoretical objections may be urged against such a conclusion. Though we have been able to obtain these organisms in 97 % + of our cases, the possibility, as suggested above, that the pneumococci so frequently present in the mouth may reach the lungs by aspiration during the death agony, or after death, during the transportation of the bodies from the wards to the morgue, is no fanciful one.[11]

The mouth and nose are frequently found at autopsy filled with fluid admixed with frothy and bloody fluid, or vomitus. It is quite evident, under such conditions, that, by mere force of gravity and the fluid communication existing between the upper and lower air-passages, micro-organisms may find their way from

[11] Because of its possible bearing upon the gravitation of fluid from the buccal cavity to the lungs, it seems worth while to describe in detail the various manipulations to which the body is subjected during the interval between death and post-mortem examination. After being formally pronounced dead by a member of the house staff, the body is rolled in a shroud, the jaw supported by a four-tailed bandage, and cotton plugged into the mouth and other orifices. Within an hour or less, the cadaver is ready for transportation to the morgue. It is lifted by the shoulders and heels, to a four-wheeled truck, which has been rolled to the bedside. During this manœuvre, with the sagging of the body the head is of necessity at a higher level than the thorax. From the ward, the body is wheeled directly to the morgue,—a distance of about 150 yards. Then again it is lifted from the truck to a wooden frame, which is placed upon the floor next to the carriage, and lying upon this, it is raised, feet foremost, to a compartment in the cold-storage chamber. Here it remains until removed to the autopsy room. In this final handling of the body, the wooden tray is slid from the compartment, the head lowest, and the body is carried upon this wooden frame to the adjacent room. The body is then rolled upon its side, abruptly jolted, and the head raised and lowered several times. The entire proceeding, therefore, involves considerable disturbance of the body before the necessary examinations are made.

the mouth to the small air spaces; or, again, by the compression of the chest wall in moving the body a false respiratory excursion may take place, causing the replacement of the air in the lungs by fluid from the mouth and trachea. If this takes place, it then seriously vitiates the value of inferences drawn from cultural findings after death, when applied to the living lung.

In order to determine the frequency of this occurrence by experimental methods, we have had recourse to rabbits, which have been given small doses of broth cultures of B. prodigiosus just before or after death. Furthermore, we have introduced broth cultures of B. prodigiosus (a half drachm or less) into the mouths of patients who had died in the wards, but before removal of the bodies to the morgue.

The method which we have used for the isolation of B. prodigiosus in the mouths and lungs of rabbits and cadavers was the following: the mouth was scraped with a sterile cotton swab; a suspension was then made in melted agar tubes, and plates with one dilution poured. The mouth plates were made in every case in order to make sure that the patients had received the culture, and thus they served as control for the lung plates. From the lung a considerable amount of expressed juice, obtained by squeezing and crushing a piece of excised lung, was distributed in melted agar tubes and pour-plates made. Both series of plates were then kept at room temperature and observed until the appearance of typical red colonies, or for several weeks. Abundant growths of various organisms were regularly obtained in these plates. In no case have we seen that the development of numerous colonies has inhibited the pigment production.[12]

In nineteen (19) cases, half-drachm or smaller portions of broth cultures of B. prodigiosus were introduced into the mouth by the interne after having pronounced the patients dead. Characteristic red colonies of B. prodigiosus developed on the

[12] A few control experiments were made, to test this point; tubes were inoculated with varying proportions of dysenteric fæces, and broth cultures of B. prodigiosus. These showed that pigment formation by this culture of B. prodigiosus was not inhibited by the excessive growth of other bacteria.

pour-plates from the mouths of these nineteen cases, and in ten cases more or less numerous colonies of B. prodigiosus developed in the lung plates. In other words, in a little over 50 % of the cases evidence was obtained that micro-organisms introduced into the mouths of patients after death enter the lungs. In half of the cases, as seen from Table V, numerous colonies developed on the lung plates.

Since it was impossible to personally control the giving of the cultures after death, we have not included in the table nine cases in which the mouth plates were negative. We, however, believe that of these nine cases some received the cultures, but that the B. prodigiosus failed to develop its characteristic color upon the mouth plates. We have positive knowledge that this occurred in one case (279, not included in table), the cotton swab of the mouth being stained pink. Moreover, as above stated, our B. prodigiosus developed pigment in over-crowded plates; nevertheless, as is well known, pigment formation is subject to a number of disturbing factors which are not readily controlled or perhaps even known.

In support of this view it may be stated that in one case, 175, abundant colonies developed in the lung plates, whereas none appeared in the mouth plates; this case has not been included in the final estimate of the percentages of positive results. For these reasons, we believe that the percentages given are too low for the mouth as well as for the lung plates.

It is certainly a striking observation that likewise in about fifty per cent. (50 %) of our forty-one cases the pneumococcus was isolated. It seems a fair inference from this coincidence that the utmost caution must be observed in the interpretation of the cultural findings of the lung from post-mortem statistics.[13]

[13] In five (5) cases, the prodigiosus culture was received shortly before the exitus lethalis. In one case (82, alcoholic delirium), numerous colonies developed in both the mouth and the lung plates. In two cases (98, 105), the mouth plates alone developed colonies, the lung plates being negative. In case 110, negative results were obtained in the mouth and lung plates; this, however, is readily accounted for by the fact that the culture was administered twenty-four and, again, fourteen hours before exitus. Another of the negative cases (C V), where no colonies developed on the lung plates, is readily enough ac-

We append below the statistics in tabulated form:

TABLE V.

TABLE SHOWING THE CASES IN WHICH B. PRODIGIOSUS, INTRODUCED INTO THE MOUTH POST-MORTEM, WAS RECOVERED FROM THE MOUTHS AND LUNGS.

No.	Mouth.	Lung.	No.	Mouth.	Lung.
166	+	—	214	+++	+
168	+	+++	224	+	—
171	+++	—	233	+++	+
172	+	+	241	+++	—
181	+++	+	258	+++	—
188	+++	++	262	+++	+
199	+	—	278	+++	—
201	+++	+++	281	++	+
205	+	—	288	+++	+++
206	+	—			

Total number of cases..... 19
Mouth positive..... 19
Lung positive..... 7

Our work upon the entrance of B. prodigiosus from the mouths of rabbits to their lungs has been unfortunately confined to two (2) rabbits. One rabbit was given 0.5 c.c. of a broth culture of B. prodigiosus when under ether. The rabbit died twenty minutes later without gasping. The body was then slanted upon a tray with elevated head. (Post-mortem, twenty-nine hours later.) No colonies of B. prodigiosus developed in either mouth or lung plates.

The other rabbit, dying from an intraperitoneal inoculation with the meningococcus, was given 0.5 c.c. of a broth culture, and died slowly several hours later. At autopsy, performed at once after death, pour-plates were made from the mouth and lungs. Numerous colonies developed on the mouth plates; the

counted for by the closure of the glottis from œdema, after a cut-throat wound of the neck. Thus, colonies of prodigiosus were obtained from the lungs in one out of three cases, 33 %, if the above reasons are valid for excluding the two cases (110 and C V). As regards these experiments, however, only negative results are of value, since one cannot make sure that the organisms introduced during life did not reach the lung after death.

lung-plates failed to show red colonies. We abandoned this line of experimentation for the reason that the conditions so obviously differ from those of the human respiratory tract, which almost constantly contains more or less fluid, at least after death.

THE AGGLUTINATING REACTIONS OF THE PNEUMOCOCCUS, STREPTOCOCCUS, AND ALLIED GROUPS OF DIPLOCOCCI.

The impracticabilty of using agglutinating reactions, especially difficult in this group of cocci, led us to forego this aid to species identification. This method, as has been repeatedly shown, when used upon species belonging to the same group, has no differential value, unless very tedious and exact quantitative serum tests are employed. For these reasons, and because of the impossibility of obtaining sera of high valency within the limited time at our disposal, we could not avail ourselves of this method during the greater part of this investigation.

Before giving our conclusions, we deem it best to give the results that we obtained upon the agglutinability of cultures of species belonging to the various groups into which we have divided the various diplococci that we encountered during the progress of our work.

We were able, however, at the end of our work, to make use of the method of obtaining mass cultures for agglutination tests described by Prof. Hiss,[14] which, in his hands, has yielded such brilliant results. With a rabbit immune serum giving a complete agglutination at 1 : 400 with the homologous pneumococcus, which we obtained through the kindness of Prof. Hiss and Dr. Borden, the following tests were made the day after receipt of the serum, which was from a recent bleeding:

Broth cultures were made of species of each of our five (5) groups of cocci, strict attention being paid to the details as given in the method described by Hiss, in the article above referred to. The results of our agglutination tests obtained with the pneumococcus rabbit serum, mentioned above, and with a normal rabbit serum, may thus be briefly described. Two diplococci of Group I were used; the first, 230, a typical pneumococcus, was obtained from a case of suppurative sphenoiditis at autopsy. This pneumococcus did not lend itself

[14] *Journal of Experimental Medicine*, 1905, vii, 223.

to the agglutination test, at least with the mass culture we employed, since the control settled out promptly, and clumps were speedily developed. The second diplococcus, C VII, another typical pneumococcus, was not clumped by the normal rabbit serum, after twenty-four hours, the control showing only a few small, microscopic clumps. With the pneumococcus serum, large flocculi developed in the 1:10 dilution, but the reaction did not become complete, even after twenty-four hours, the fluid still being cloudy. Small flocculi were, however, found in the 1:200, as well as in the 1:1600 dilution, unlike the control.

Only one diplococcus of Group II was tried, namely 71, a typical Streptococcus mucosus, which gave a slight reaction only in the lowest dilution, namely, 1:10. Normal rabbit serum gave no reaction in a similar dilution.

One diplococcus of Group III was tried (199). It gave with the pneumococcus rabbit serum, a positive reaction at 1:100, but no complete reaction was obtained even at 1:10, the fluid becoming only slightly clearer with small clumps on the sides of the tubes. The reaction in the 1:10 and the 1:100 dilutions differed only in the size of the clumps. Normal rabbit serum had no action upon this diplococcus.

Three organisms of Group IV were tried. Culture 49 a gave a slight reaction with pneumococcus serum, in the 1:10 dilution, the clumps being larger than those of the control. After twenty-four hours, all the dilutions and the control, however, became practically clear. Culture 149 was not affected by the pneumococcus or the normal rabbit serum in any dilution. With culture C II the dilutions and the control were practically alike, although possibly there were larger granules in the lowest dilution than in the control.

Two organisms of Group V, typical streptococci, were tested. Culture 214 S did not lend itself to the agglutination test on account of the prompt development of large granules, and the rapid clearing of the fluid in the tubes in the control, as well as in dilutions. With the other streptococcus, 153, larger granules were found, and a quicker settling in the lower dilutions occurred than in the control, which, however, after twenty-four hours, became perfectly clear.

Thus, if one may judge from a single examination, the method of mass cultures or agglutination tests have shown that only in the case of the pneumococcus, C VII, positive agglutinations were obtained, and even with this organism no complete reaction was given in the lowest dilution, namely, 1:10, after twenty-four hours, with the possible exception of the diplococcus 199, of Group III.

It seems to us somewhat forced to draw any conclusions, from the single agglutination test described above, upon the method of using mass cultures, but we believe that the test shows that the diplococci which we have not identified as pneumococci—in other words, all those belonging to other groups than Group

I—have no or very slight agglutinating affinities to the pneumococcus.

Before concluding, it may be well to state that we fully recognize the arbitrariness of our method of classifying the diplococci belonging to the pneumococcus and streptococcus groups.

The experience we have gained in the examination of a large number of cultures convinces us that it is not justifiable to consider all diplococci which ferment inulin, pneumococci. Thus, C II and 149 and 49a are rapid inulin fermenters, but in all other respects they resemble streptococci. It is of interest to note in connection with 49a, that its property of fermenting inulin is a most variable one. Thus, on passage through the first mouse, after its primary isolation from the lungs upon glycerine-agar, it lost its fermenting action upon inulin, only to regain it on further cultivation upon serum water made from the same stock of inulin. Further passages through mice did not affect this function. Again, later cultures of 49a lost their capacity to ferment inulin, but regained it on transplantation, and are now active and rapid fermenters. Cultures C II and 149 have never changed, having always been active fermenters.

We consider that the presence of capsules like the inulin reaction can not be regarded as an infallible guide for the differentiation of these diplococci, especially when the Hiss capsule methods are employed, for the reason that we have encountered streptococci with distinct capsules.

The cocci of what may be called the intermediate groups, III and IV, it may be of interest to state, have been found in the blood during life, and have been recovered from the pial exudate of cases of meningitis. A further study of these interesting diplococci is greatly needed.

CONCLUSIONS.

The following conclusions may be drawn, based upon the result of our researches:

1. Organisms of the pneumococcus or streptococcus group are present in the lungs of practically all cases, whether normal or

showing a variety of lesions; strictly speaking, they were found by us in forty out of forty-two cases, or in 95 % of our series.

2. The pneumococci and the streptococci were obtained in practically similar percentages—that is, in 50 % of the cases.

3. Pneumococci were not obtained more frequently in the small series of patients exposed for some time to hospital atmosphere; our tables show the contrary to obtain. The number of cases examined were, however, insufficient, and the findings may thus be accidental, and hence of no value.

4. Test micro-organisms, namely, small portions—half a drachm or less—of B. prodigiosus, introduced into the human mouth after death, were conveyed to and recovered from the lungs by culture in a little over half of the cases in which this experiment was tried. The test micro-organisms are, we believe, conveyed to the lungs with the fluid which collects in mouths of persons after death, and which in many cases collects just before death. The numerous manipulations entailed in the removal of the body from the wards to the morgue greatly facilitate the entrance of any fluid from the pharynx and buccal cavity into the lungs.

It follows logically, from the results obtained in this experiment, that the cultural findings after death are no guide to the bacterial contents of the lungs during life, and that any deductions made from such findings are unreliable and deceptive. Granted that our explanation be correct, there is every reason to believe that any of the micro-organisms present in the mouths and pharynx and in many cases in the stomach contents may enter the lungs and, if the conditions be suitable, increase in numbers, during the time between death and the examination of the lungs.

5. There exists, perhaps, more frequently than has hitherto been suspected, a series of diplococci, intermediate between the typical pneumococci and streptococci. The diplococci of this type have been found in forty (40) per cent. of our cases.

The differential diagnosis of these atypical diplococci from the pneumococci and streptococci is a difficult one, depending, as it does, upon general cultural characteristics. No single character, such as the presence of capsules or the fermentation of

inulin, virulence, etc., has been found to be a certain criterion. The few agglutinative reactions we have made seem to show that these intermediate diplococci, those of Groups II, III, and IV, have no or only slight agglutinative affinities to the typical pneumococcus. Further tests must, however, be made with the various methods at our disposal before this statement can be accepted as final.

These diplococci are of interest from the fact that they have been found in the blood during life, and in the pial exudate of cases of meningitis, endocarditis, etc.

6. Our studies have thrown no light whatever upon the conditions which determine the onset of lobar pneumonia in apparently healthy persons. Moreover, we have been unable to draw conclusions as to the presence of pneumococci in the lungs during life, or as to the channels by which they gain access thereto.

In concluding, we gladly avail ourselves of the opportunity of acknowledging our indebtedness to Prof. T. Mitchell Prudden for help and advice freely given in the course of this work, and to the various members of the house staff of Bellevue Hospital for their aid and assistance in the pursuit of these researches, especially to Dr. Frank Erdwurm.

STUDIES ON THE PNEUMOCOCCUS.

By CHARLES W. DUVAL, M.D., and PAUL A. LEWIS, M.D.

(*From the Pathological Laboratory of the Boston City Hospital.*)

Plate XXIX.

The work on the pneumococcus at the Boston City Hospital, under the auspices of the Commission for the Investigation of Acute Respiratory Diseases, of the Department of Health of the City of New York, was begun November 1, 1904, and continued until July 1, 1905. The director of the hospital laboratory, Dr. F. B. Mallory, encouraged us to accept the proposals of the Commission, placed the facilities of the laboratory at our disposal, and has maintained a constant interest in the progress of the investigation. The physicians of the hospital's clinical services, both visiting staff and house physicians, have given us every assistance in procuring material. On the part of the Commission we have had the support and advice of Dr. Theobald Smith. The photomicrographs we present were taken for us by Dr. S. B. Wolbach. To all of these gentlemen we extend thanks for their interest in the work and their invaluable assistance.

The purpose of the investigation was to ascertain, so far as possible with the material under our control, the distribution of the pneumococcus, and by a subsequent comparative study of cultures isolated to see if there might be a constant variation in any character or set of characters coincident with the source of origin. We wish to report as concisely as possible the data which bear directly on these questions.

One of the constant difficulties which has confronted us throughout the work has been the separation of the pneumococcus from Streptococcus pyogenes. The inulin test of Hiss having been proposed as an absolute method of differentiating these two species, we give in detail our experience with it in the form in which it was originally proposed and in the modification we find most satisfactory.

In the course of the work we have twice encountered a diplococcus which has proved identical in general characters with those isolated by Howard and Perkins,[1] and by Richardson[2] in this country and by several observers abroad. It has usually been described under the name Streptococcus capsulatus or mucosus. As we have been able to add some new facts to those previously reported, we give our experience with it in some detail.

Except for the method of using the inulin, our technique is a combination of methods previously in use and well known to investigators who have studied this group of bacteria. We therefore make the briefest possible statement of it.

METHODS.

In cultivating the pneumococcus we have found that attention to the composition and reaction of the culture medium is essential. All media having bouillon as a base should be made from beef. The reaction should be adjusted with but one change, if possible, to between 0.4 and 0.8 % acid to phenolphthalein (cold titration). Dextrose, or some other carbohydrate fermentable by the particular culture in question must be present in quantity of from 0.5 to 1 %, or growth will be neither constant nor abundant. The addition of from 2 to 5 % of "fresh" defibrinated blood to dextrose agar-agar we have found to make the most satisfactory medium for routine cultivation. If these conditions are fulfilled, the temperature has only its usual influence on rapidity of growth. Growth always takes place at room temperature. The gelatin-agar mixtures with the addition of dextrose give good growth. The other culture media which we have found particularly useful for cultivation and identification of the organism are litmus-milk and the serum-water-sugar mixtures made according to the formulæ of Hiss. The lactose-serum water is particularly useful. It is coagulated by both the pneumococcus and the streptococcus as a rule, but by the former in twenty-four hours, and by the latter only after many days. It cannot be considered to be an absolutely differential medium.

In making isolations we have placed our dependence on the plating method with surface seeding. Dextrose agar-agar plates are poured and solidified. Several drops of sterile defibrinated blood are scattered over the surface with a finely drawn pipette. The particular kind of blood used seems to be unimportant. With a sterile platinum loop, several drops of a previously made bouillon or salt-solution suspension of the material to be plated are added to one of the drops of blood on the plate. After mixing thoroughly with the loop, a drop or two are carried to a second drop of blood, and after mixing again, to a third. After these dilutions are accomplished, the drops are spread as evenly as possible over the plate. If the agar-agar is firm (1.7 to 2 %), and the wire loop soft, this can be accomplished without roughening the surface of the

[1] *Jour. of Med. Research*, 1901, vi, 163.

[2] *Jour. of the Boston Soc. of the Med. Sciences*, v, 499.

medium. Colonies to be studied are transferred from the plates to the dextrose blood-agar. The isolation from the heart's blood of an animal after subcutaneous or intraperitoneal inoculation of the material to be examined is less satisfactory as a routine method, in our experience. If used with discretion, the method does not fail, but it is more tedious and expensive. We will say more of the method in discussing the virulence of the pneumococcus.

The identification of the pneumococcus cannot be made by any one feature or test. It is best made within a few days after isolation of a culture. The capsule is then more certain to be large and distinct, and the lanceolate diplococcus form is more marked. As the period of cultivation grows longer, the capsules are less constantly demonstrated. The cocci become smaller and the pairs more flattened. Under certain conditions which we do not fully understand, but which seem to be generally unfavorable to active growth, the most typical pneumococcus culture may grow as pairs of oval and flattened cocci in long chains. Many individual cocci in such a chain will appear distinctly biscuit-shaped. Figure 4, however, is intended to demonstrate that very long chains do occur even under favorable conditions with freshly isolated pneumococcus cultures. In such cases the size of the capsule, the larger size of the individual coccus, and the shape of the pairs must be depended on for the diagnosis.

The colony on blood-agar varies under conditions we have not been able to control, from an almost invisible dry, flat colony, or a raised, moist, or dewdrop-like growth, to a dead white, viscid colony 2 mm. in diameter, resembling that of Staphylococcus albus. Not much dependence can be placed on such a variable character in identifying a culture.

Culturally the rapid fermentation of lactose in serum water, the rapid coagulation of litmus milk, and the fermentation of inulin are the points on which most dependence must be placed, although any one of these characters may be absent with a given culture. If inulin be fermented by a micro-organism having in general the characters of the pneumococcus, the identity is established, in our experience. That there are, however, non-inulin-fermenting pneumococci, we feel certain. Such cultures will be described when we discuss the inulin test.

OCCURRENCE AND DISTRIBUTION OF THE PNEUMOCOCCUS.

In the study of the distribution of the pneumococcus, material was studied from:

I. Autopsies in the hospital.
II. Surgical material sent to the laboratory.
III. Cerebro-spinal fluid obtained by lumbar puncture.
IV. Sputum of—
    A.—Pneumonia cases.
    B.—Other cases with bronchial or pulmonary trouble.
    C.—Saliva from persons in good health at work in the hospital.

I. *Autopsies.*—The efforts of this laboratory have been chiefly directed to the study of autopsy material. We will report the

results of the bacteriological examination of the lungs of thirty-three persons dying with acute lobar pneumonia. We include also the bacteriological report of examination of the lung in four cases in which death was ascribed to lesions outside the lung, although the lungs showed areas of broncho-pneumonia, and in two cases in which there was no evidence of pulmonary disease.

In order that the value of the results may be correctly estimated, some explanation is necessary. Early in the course of the work our only aim was the isolation of the pneumococcus. Thus all of the examinations reported bear on the question of the general distribution of the pneumococcus. All of the cases in which the pneumococcus was the only bacterium isolated from the lung belong to this period when we were attempting nothing but its isolation. No consideration should be given to the negative aspect of these cases.

When the local distribution of the pneumococcus in the lung is considered, only two of our cases are of importance. These are reported in detail (Autopsy 1905, 47, and Autopsy 1905, 82).

The reported data which have reference to the association of bacteria in the pneumonic lung are not complete. Other micro-organisms were found which were only roughly classified and noted. Especial effort was later made to isolate the streptococcus. In general, the negative character of the findings has no significance. There is one particular exception. The influenza bacillus has been kept constantly in mind and our technique has been suitable to grow it. We have, however, met with it only once (Autopsy 1905, 82), and the detail of that case is given.

The facts which concern the general distribution of the pneumococcus, the constancy with which it occurs in the pneumonic lung, its association with other bacteria in the pneumonic lung, and its presence in the lungs of persons dying of other causes than acute lobar pneumonia are presented in the following table:

(*a*) Cases in which the pneumococcus alone was isolated from the lung:

Acute lobar pneumonia	5 cases.
Acute pericarditis	2 cases.
Acute meningitis	1 case.

The cases of pericarditis and meningitis showed areas of broncho-pneumonia.

(*b*) Cases in which the pneumococcus was isolated together with the streptococcus:

Acute lobar pneumonia..... 22 cases.
Wood-alcohol poisoning..... 1 case.
Chronic interstitial nephritis..... 1 case.

The cases of poisoning and of nephritis showed no lesion of the lung.

(*c*) Cases in which the pneumococcus was isolated together with: (1) Streptococcus pyogenes and (2) Pneumo-bacillus of Friedländer:

Acute lobar pneumonia..... 4 cases.
Cerebral hemorrhage with broncho-pneumonia. 1 case.

(*d*) Cases in which the pneumococcus was isolated together with: (1) Streptococcus pyogenes, (2) Staphylococcus aureus and albus, (3) Influenza bacillus, (4) Pseudo-diphtheria bacillus:

Acute lobar pneumonia..... 1 case.

(*e*) Cases in which the pneumococcus was isolated together with: (1) Streptococcus pyogenes, (2) Pneumo-bacillus of Friedländer, (3) Streptococcus mucosus:

Acute lobar pneumonia..... 1 case.

After the work was well under way, it became apparent that the pneumococcus was present with great constancy in the pneumonic lung, and that it often occurred in the healthy as well as in the involved portions of the organ, usually in association with the streptococcus. It was realized that more careful study of the localization of pneumococci and streptococci in the lungs of pneumonia cases was essential to a consideration of the relationship of these micro-organisms to the disease and to one another. To answer these questions the examination of material from the lungs of patients recently dead was necessary. Decisive data might be expected from cases in which death occurred in an early stage of the disease. In the latter part of the winter we have had two cases in which these conditions were approximated. The records are reported in detail.

One of these cases (Autopsy 1905, 82) was unusual in that B. influenzæ was present in very large numbers. From its peculiar interest in this respect the clinical history is reported.

Case I.—*Acute lobar pneumonia.* Autopsy 1905, 47, was performed nine hours after death. There was late red and early gray hepatization of the right lower lobe. All other lobes seemed normal. Twenty-six plates were made from the upper, middle, and lower parts of each lung; 1 % glucose-agar with rabbit's blood was used and the plates were seeded on the surface. Suspensions of same number of loops of material from each portion of the lung were employed.

All the plates showed some colonies of Staphylococcus aureus and all showed the pneumococcus and the streptococcus in about equal numbers as estimated by the microscopic examination. Colonies were transplanted and cultures identified as shown in the accompanying table.

Lobe of Lung.	Colonies.	
	Pneumococcus.	Streptococcus.
Pneumonic right lower lobe.....	7	4
Healthy right upper lobe.....	3	5
" right middle "	5	3
" left lower "	2	3
" left upper "	3	2

Case II.—E. H. B., aged forty-five, was admitted to the Boston City Hospital, May 3, 1905. Clinical diagnosis: lobar pneumonia. On admission the patient was delirious. A member of his family stated he had been sick for one week. There was considerable prostration, marked cyanosis and dyspnœa. He was delirious and required restraint. The eyes, nose, throat, and ears were negative. The chest was symmetrical. The heart sounds were indistinct and a friction rub was heard over the base of the præcordium. The left lung revealed dulness from the angle of scapula to the base, extending into the axilla. The spleen was not felt. The abdomen was tympanic but not tender. The temperature on admission was 104°, the pulse 135 and respirations 40. The patient remained delirious, requiring restraint. His condition became progressively worse and death ensued on the second day after admission.

Autopsy 1905, 82, was performed seven hours post-mortem. Anatomical findings: Acute lobar pneumonia; acute pleuritis; acute splenitis; chronic pericarditis; congestion and œdema of lungs. Gross examination showed a well-marked consolidation of the left lower lobe. On section the lower two-thirds of the pneumonic lobe were found in the stage of gray hepatization. The upper third was moist, granular, and dark red in color. The pleura was bathed with a fibrino-purulent exudate. The left upper lobe showed congestion and œdema. The right lung was negative. Duplicate smear-preparations were made from all the lobes. One of each was stained by Gram's method and counter-stained with pyronin. The duplicates were stained for capsules. All of the lobes showed encapsulated diplococci. The smears from the pneumonic lobe showed comparatively few diplococci contrasted with the number from the normal lobes. In all the smears there were phagocytic cells which contained from two to sixteen pair of Gram-positive lanceolated diplococci. Still other phagocytic cells contained innumerable Gram-negative bacilli. Here again the greater numbers were observed in smears made from the uninvolved lobes.

A series of twenty-five plates was seeded, five plates of uniform seeding representing each lobe. All plates developed colonies of the pneumococcus, Streptococcus pyogenes, B. influenzæ, B. pseudo-diphtheriæ, Staphylococcus albus and aureus. The number of colonies of pneumococcus and B. influenzæ was greater on the plates representing parts of the lung other than the pneumonic area. The colonies of B. influenzæ ranged from one to three hundred per plate. Though all the plates developed colonies of Staphylococcus aureus, no one plate contained more than fifty. The number of colonies of pseudo-diphtheria bacillus was relatively small. Plates were also made from the heart's blood (1 c.c. of blood being suspended in 10 c.c. of dilutant). Here again developed pneumococcus, streptococcus, B. influenzæ, B. pseudo-diphtheriæ, and staphylococcus. A few plates contained so many colonies of B. influenzæ that it was impossible to determine their number.

From the study of these two cases no conclusions are warranted.

II. *Surgical Material.*—The results of the examination of surgical material can be summarized briefly.

Pneumococcus present in pure cultures:

Alveolar abscess	1 case.
Abscess in groin	1 case.
Abscess in chest	1 case.
Pelvic abscess	1 case.
Septic knee	1 case.
Septic uterus	2 cases.
Acute mastoiditis	2 cases.

Pneumococcus and streptococcus:

Abscess of shoulder	1 case.

The study of pleural exudates deserves special mention. Twenty-five examinations have been made.

Pneumococcus alone	9 cases.
Pneumococcus and streptococcus	6 cases.
Streptococcus alone	10 cases.

It is noteworthy that in the early examinations it was considered sufficient to identify either the pneumococcus or the streptococcus, and no consistent effort was made at any time to grow both the pneumococcus and the streptococcus.

III. *Cerebro-Spinal Fluid.*—From the cerebro-spinal fluid taken by lumbar puncture from two cases, the pneumococcus was isolated in pure culture. The material was sent from outside the hospital and no subsequent history has been obtained.

IV. *Sputum.*—By the plate method the pneumococcus has been isolated from the sputum of several cases of pneumonia.

Examinations of sputa from cases of chronic bronchitis and suspected tuberculosis have shown the presence of the same organisms. The pneumococcus was isolated eight times from tubes inoculated for the routine examination for diphtheria bacilli.

Saliva from the mouths of twenty-four individuals in the laboratory and hospital was examined. These people had never had pneumonia and were in perfect general health at the time of examination, no account being taken of the slight chronic lesions of the pharynx with which most people in this climate are affected in the winter months. Pneumococci were easily isolated in every instance. In this series of examinations no consideration was given to cultures which did not ferment inulin.

Saliva was suspended in bouillon and plated as above described. Colonies were transferred to blood-agar tubes. Stains were made from the growth twenty-four hours old by Gram's method and usually by methods for identification of capsules. If the morphology and growth were characteristic, the cultures were at once tested in inulin bouillon and, as before stated, only those producing a marked acidity, shown by titration, have been regarded as pneumococci. As there is no evidence that the streptococcus ever ferments inulin, these points seem sufficiently conclusive for the present purpose. Inulin-splitting pneumococci we conclude are present during the winter season in the saliva of practically every person living under ordinary city conditions.

Our studies on the distribution of the pneumococcus have shown that it is to be found practically always at autopsy in the lungs of people dying with lobar pneumonia, and that it is found in the healthy as well as in the diseased portions of the lung. At times this micro-organism is found in the non-pneumonic lungs of persons dying from causes other than pneumonia. Very frequently the pneumococcus is found in purulent affections of the serous cavities and in acute abscesses of other parts. In the mouth and throat of persons in good general health it is found so often that its presence can have no pathological significance.

It is further evident that Streptococcus pyogenes and the

pneumococcus are found in much the same places and conditions and very often occur together. The facts of general distribution hardly warrant the supposition that the one bacterium has a closer etiological relation to acute lobar pneumonia than the other. A more extended and careful study of the local distribution of these two organisms in pneumonic lungs might, however, lead to valid negative or positive conclusions on this most important point.

CHARACTERISTICS OF THE PNEUMOCOCCUS IN RELATION TO ITS SOURCE OF ORIGIN.

In attempting to draw distinctions between pneumococci of various sources we have met with no success. We have paid most attention to cultural features. The fermentation of inulin characterizes a definite group of pneumococci. Inulin-splitting pneumococci are found with frequency among those cultivated from all sources. We have found no other cultural feature which is more distinctive.

We have also considered the virulence of cultures to some extent. We have tested the virulence of thirty-five recently isolated cultures, in large part from the pneumonic lung. We have used young rabbits. The dosage has been either 10 c.c. of a twenty-four-hour glucose-bouillon culture showing heavy growth or the whole of a twenty-four-hour culture on glucose agar plus rabbit's blood. In some instances two or three such agar cultures have been given. The injection has usually been made into the peritoneum. For control, animals have been injected subcutaneously and intravenously. In only one instance has a fatal result been attributable directly to the culture; the organism was isolated from the saliva of a healthy person. We do not consider our work on these points sufficiently extensive to be entirely conclusive; so far as we have gone, our cultures from all sources have been identical in possessing a low grade of virulence towards laboratory animals and in showing no cultural variation coincident with their source.

As bearing on the results of previous workers who have written

on the virulence of the pneumococcus, we offer the following observations. Depending on the plate method for isolation, we have frequently inoculated into animals the original material, namely, sputum, pus, or suspensions from the lung. The results of these inoculations fall into two distinct classes. In several instances the pneumococcus was present in pure culture as estimated by smear preparations and plate culture. In each of these cases the animals survived the injection of large quantities of material. One young rabbit received 5 c.c. of pus from the base of the brain in a case of pneumococcus meningitis. The examination of smear preparations showed large numbers of encapsulated pneumococci in a good state of preservation. Two of these animals still live. Two were killed on the fifth day and the pneumococcus was recovered from their blood.[3]

In the other class of cases the material examined contained a mixture of bacteria. The injection of small or moderate doses gave in most instances a fatal result in from one to four days. The pneumococcus was always found in the blood, generally in pure culture. If bacilli belonging to the Friedländer group were present, they were frequently found in the blood of the inoculated animal, especially when the guinea-pig was used. In view of the series of experiments with material containing the pneumococcus only, and the consistently negative results with pure cultures, we doubt whether the presence of the pneumococcus in the blood of these animals indicates that it alone is responsible for the death of the animal.

It seems probable that when the blood of the animal first inoculated is transferred directly to another animal, a false impression of the virulence of the culture finally recovered may be given, because of the exalted virulence attained by the direct passage from animal to animal.

THE ACTION OF PNEUMOCOCCI ON INULIN.

Early in the year we placed great reliance on the inulin test as an absolute means of differentiating the pneumococcus from

[3] The culture isolated from the rabbit inoculated with pus from this case of meningitis is described in more detail in our discussion of the non-inulin-fermenting pneumococci.

Streptococcus pyogenes. We used then the serum-water-inulin medium proposed by Hiss. Several hundred tubes of the serum-water medium made with inulin ("Merck") and beef-serum gave consistent results with ten pneumococcus cultures and several cultures of Streptococcus pyogenes.

These cultures have been retained and have served as types with which our organisms isolated later have been compared. Except for the one lot of inulin-serum-water medium mentioned, which fortunately was large, we have had nothing but failure in its use. This failure we have been unable to explain satisfactorily. We do not feel convinced that the medium is essentially faulty, but there has been perhaps some disturbing factor in our material or method of preparation which we have not been able to discover or control. We have, however, had no trouble in obtaining constant results with the pneumococcus in serum-water media containing other carbohydrates.

In trying to explain the inconstancy of our results we have proceeded as follows: The reaction of the medium has been carefully controlled and varied from neutral to 1 % acid to phenolphthalein with differences of .1 % of acid. These differences bring about distinct variations in the opacity of the preparation. But when inulin is added no one degree of acidity is found to give more consistent results than another. Inulin has been sterilized separately and added to the tubes with a pipette, but the results have been no better. We have worked with the following preparations of inulin:

Merck's inulin, white.
" alant starch, Lot I.
" " " white, Lot II.
" inulin ("highest purity").
Bausch and Lomb's inulin, white.

The preparation which gave the one consistent result was Merck's, Lot II. Repeated trials with the same lot have failed. The two lots of Merck's white inulin and Bausch and Lomb's white inulin when tested in 10 % solution in distilled water with Fehling's solution have shown reducing substances present in quantity equivalent to from 2 to 3 % of dextrose in the dry

substance. If a 10 % solution in distilled water is subjected to sterilization in the autoclave (at 10 lbs. pressure for ten minutes), the percentage of reducing substances may be increased to five. These reducing substances may not be dextrose and they may indicate the presence of other impurities which do not reduce the copper. One small quantity of inulin (Kiliani) obtained from Bausch and Lomb was subjected to the autoclave before the possibility of injury had suggested itself. Subsequently it was found to contain about the same percentage of the reducing substances as that shown by other preparations. This preparation we have not been able to buy again.

INULIN BOUILLON.

In the hope of avoiding the difficulties encountered in the use of the inulin-serum-water medium, at the suggestion of Dr. Theobald Smith we undertook a series of experiments with inulin in bouillon. The outcome exceeded our expectations. The results of successive titrations remained uniform, and we can recommend this modification of the inulin test for routine work. Using these results as a basis, pneumococci can be classified as follows:

A. Inulin fermenters
- I. High acid producers.
- II. Medium acid producers.
- III. Low acid producers.

B. Inulin non-fermenters.

Bouillon is made according to standard methods from beef. It is reduced in acidity to from .2 to .4 % normal to phenolphthalein. Subsequent sterilization restores the acidity to from .5 to .8 %. Carefully cleaned tubes containing 9 c.c. of bouillon are sterilized. A 10 % solution of inulin in distilled water is sterilized in the autoclave at 15 lbs. pressure for fifteen minutes, and with a sterile pipette 1 c.c. of the inulin solution is added to each tube of bouillon.

The tubes are simultaneously inoculated with the cultures to be tested, incubated for the period of the experiment, and then steamed for a short time in an Arnold sterilizer. They are then titrated for percentage acidity, using the same solution and in-

dicator. Theoretically it might be better to prepare as a culture base sugar-free bouillon. It has proved satisfactory, however, to use as control cultures in bouillon containing no inulin.

The table on page 486 shows the result of titrations in two lots of inulin-bouillon made from different material and different brands of inulin. The period of growth in this experiment is one week. With a little experience the high and medium acid-producing organisms can be distinguished in twenty-four hours, and the low acid-producing organisms in forty-eight hours, although the numerical differences at these periods are small and inconstant. It is probable that if the number of cultures worked with were doubled or tripled, organisms intermediate between these groups and types might be found, but the types are sufficiently marked to illustrate the efficiency of the method employed. With two or three exceptions, all of our inulin-fermenting organisms that have survived prolonged cultivation appear in the table. The non-inulin-fermenting group might be greatly enlarged by the addition of about thirty cultures which we still retain. As the differentiation of many of these cultures from Streptococcus pyogenes is difficult and uncertain, we prefer to test only those whose identity rests on secure ground.

The classification is based on the reactions of cultures which have been several weeks under cultivation. We have recently made a number of titrations with freshly isolated cultures. Many of them have given reactions of from 5.8 to 6.1 % of a normal acid solution at the end of a week. We should hesitate, therefore, to separate the two cultures of Type I from those of Type II on this basis alone. As they are the only cultures among our stock which ferment mannit, and as their titration is always somewhat higher than that of any other culture, we feel that the distinction is justified. The cultures of Group A we think are all pneumococci.

Group B requires explanation. By comparison of the figures given for the plain bouillon controls with those given for the members of Type IV, it is seen that the average of the latter is somewhat higher. This is probably due to the presence of small quantities of fermentable impurities in the inulin. Inspection

GROUP A.= Inulin Fermented.

Type.	Culture Number.	Total Acidity. Bouillon. Plain.	Inulin Lot I.	Inulin Lot II.	Average.	Remarks. (Uninoculated Controls Titrate at 0.6 to 0.8 %.)
I High Acid Producers	C.D. 16	1.1 %	6.5 %	6.8 %	6.57 %	These cultures ferment mannit.
	C.D. 12	1.0 %	6.6 %	6.4 %		
II Medium Acid Producers	C.D. 9	.9 %	4.3 %	4.3 %	4.53 %	The cultures of this type and also those of Type I are distinguished from Type III and the non-fermenters by the fact that they produce a diffuse cloud and heavy sediment in the bouillon, recognizable in from 16 to 24 hours.
	C.D. 19	1.0 %	4.3 %	4.2 %		
	C.D. 20	1.1 %	4.2 %	4.6 %		
	C.D. 21	1.0 %	4.9 %	5.0 %		
	C.D. 22	.9 %	4.5 %	4.8 %		
	C.D. 23	.9 %	4.5 %	4.6 %		
	C.D. 24	1.1 %	4.8 %	4.5 %		
III Low Acid Producers	C.D. 1	1.0 %	2.6 %	2.6 %	2.6 %	These cultures are also to be distinguished from the non-fermenters by a slight diffuse clouding of the bouillon which is marked on the second day of cultivation.
	C.D. 2	1.1 %	2.3 %	2.7 %		
	C.D. 4	1.1 %	2.8 %	2.3 %		
	C.D. 6	1.2 %	2.6 %	2.4 %		
	C.D. 8	.9 %	3.3 %	2.2 %		
	C.D. 10	1.0 %	2.6 %	2.7 %		
	C.D. 18	1.1 %	3.3 %	2.6 %		
	C.D. 25	.9 %	2.0 %	1.9 %		
	C.D. 26	1.1 %	3.0 %	3.1 %		

GROUP B. = Inulin not Fermented.

IV	C.D. 7	.9 %	1.4 %	1.5 %	1.65 %	These cultures have been fully identified. The complete description of C.D. 7 appears in the text, page 487. This group could be greatly enlarged, but cultures might be introduced that could not be certainly differentiated from Streptococcus pyogenes. See page 485.
	C.D. 27	.8 %	1.6 %	1.4 %		
	C.D. 14	1.0 %	1.1 %	1.5 %		
	C.D. 28	1.1 %	1.6 %	1.7 %		
	C.D. 29	.9 %	1.7 %	1.2 %		
	C.D. 13	1.0 %	1.4 %	1.3 %		

Note: Streptococcus pyogenes cultures titrate up to 1.6 % acid.

of the titrations with this group shows that the method gives no aid in the identification of these cultures. The cultures included in the table, judged by all of their characters, are without doubt pneumococci. We believe, however, that some cultures which according to the titration with inulin bouillon belong in this group are typical streptococci. It is not our purpose at present to classify the cultures of this group. In order to make clear our point, that there are pneumococci which do not ferment inulin, we give the following description and history of such a culture.

Culture C. D. 7.—Isolated by plate method in December, 1904, from the lung of a case of acute lobar pneumonia. A large number of colonies were examined, but no inulin fermenter was found. C. D. 7 was at that time a Gram-positive diplococcus. The lanceolate pairs were surrounded by a large capsule which could be easily stained by either of the Hiss methods. The capsule persisted for some weeks in culture, but was finally lost. The colonies on Loeffler's blood-serum and rabbit's blood-agar were large and moist. Growth in glucose-bouillon was profuse, the medium being diffusely clouded. Litmus milk was coagulated in twenty-four hours, and in forty-eight hours the litmus in the lower half of the tube was decolorized. Lactose-serum water was coagulated in twenty-four hours.

At the present time (June 20, 1905) these characters, excepting the capsule, are well preserved. Glucose-bouillon is still diffusely clouded, and a Gram stain made from a bouillon tube shows a predominant number of rather asymmetrical pairs of large lanceolate cocci. There are many chains, but they are all short, containing from four to ten cocci. The morphological characteristics are intact, and as well marked as at the time of isolation. This culture has never coagulated the serum-water medium, and in our titrations with inulin-bouillon its total acidity has never gone above 1.5 % acid.

Although we have given to few cultures among the non-fermenting group so close attention as we have to C. D. 7, we are certain that others of these cultures are pneumococci and that in routine examinations the non-inulin-fermenting group will be frequently encountered. Since the introduction of inulin has made the identification of some groups of pneumococci relatively easy, the difficulties previously encountered in distinguishing this organism from Streptococcus pyogenes are significant only in the non-inulin-fermenting group. The cultures which ferment inulin, and a few of those which do not, have the pneumococcus characters so well developed and preserve

them so constantly in culture that their identification by means formerly available is not very difficult. On the other hand, it is probable that careful study of the non-fermenting group will establish intermediary varieties and may render unavoidable the conclusion that the pneumococcus and the streptococcus are races of a single species of bacterium. That the pneumococcus and streptococcus are not the extreme types of such a species seems not improbable if one takes into consideration the saprophytic streptococcus on the one hand and such cultures as the encapsulated diplococcus of which we will give an account on the other. There may also be varieties of the species which have been separated even farther from the usual types. For example, we will give in detail the record of a culture previously mentioned:

Culture from case of cerebro-spinal meningitis (Autopsy 1905, 33).—Stained preparations from the pus at the base of brain showed large numbers of encapsulated diplococci staining by Gram's method. Cultures on Loeffler's blood-serum and blood-agar were negative so far as micro-organisms other than pneumococci were concerned. There was no growth on the surface, but careful examination of the condensation water showed a few encapsulated Gram-staining diplococci. Subcultures were negative. Five cubic centimeters of the pus at the base of the brain were inoculated into the peritoneal cavity and subcutaneous tissues of a rabbit weighing 750 grams. After twenty-four hours, the animal ate well and had lost no weight. On the fifth day it was killed. Examination of the heart's blood showed a few lanceolate diplococci retaining Gram's stain and possessing a distinct capsule. Cultures on Loeffler's blood-serum were negative. In the condensation water of blood-agar tubes, a few diplococci agreeing in negative and positive character with those from the original material were found. After repeated and frequent subcultures covering a period of six weeks, during which time proliferation was very slight and confined to the condensation water, the diplococcus rather suddenly increased its activity of growth. It grew fairly well in fine colonies on the surface of blood-agar, but did not grow on plain agar or on Loeffler's blood-serum. In milk it produced a slight acidity after several days. No fermentation of sugars could be demonstrated in the serum-water-media. After two or three generations of this relatively abundant growth its activity again declined, and in spite of repeated efforts the culture died out. Cultivation failed though the reaction and sugar content of media were varied and blood and serum were added.

The organism just described was evidently a pneumococcus which differed very widely from the members of this group usually encountered. It had morphological characteristics which,

as shown in Fig. 5, were sufficiently definite to classify it as pneumococcus. Perhaps one could conclude from the experience with this culture that there are parasitic pneumococci so delicate as to be unrecognized by our present methods of cultivation and at the same time possessing possibly pathological significance.

It might be said that the method which we propose for using inulin is too tedious for routine work. To obviate this difficulty, we have made control observations with inulin-bouillon made in bulk and sterilized after tubing. The quantity in each tube need not be the same, and the medium is as efficient as that more carefully made. The inulin when sterilized in the bouillon does not undergo sufficient reduction to injure the efficiency of the test. The time we have used for the reported tests (one week) is necessary to bring out the details of grouping which we have described, but, as has already been mentioned, organisms belonging to different groups can be roughly separated in a shorter time. Neither is the titration a necessity; the medium and high acid-producing subdivisions cause in the inulin-bouillon a diffuse cloud and heavy sediment which are apparent in from sixteen to twenty-four hours. The low acid-producers show a well-marked diffuse cloud in the medium on the second day. The non-fermenters never cloud the bouillon and the sediment of bacterial growth remains slight. Though titration is not necessary for the recognition of the fermenting pneumococci, it has served to distinguish finer differences between groups, and as a routine optical method the bouillon has all the advantages of the serum-water medium. We think that the group of low acid-producers cause coagulation irregularly, because the total acidity which they produce under unfavorable conditions is often just sufficient to change litmus, and this degree of acidity seems to approximate the coagulation point of the serum-water. The inulin-bouillon has the added advantage that its constituents are easily procured and combined, its constitution is more uniform, and the reaction produced is more delicate when estimated by titration for percentage of acidity than is the case with the serum-water medium.

We have retested the samples of inulin before used, and find that when used in the bouillon the brands labeled "Highest purity," which are hard to secure, are not essential. All the samples of white inulin mentioned before and one lot from another supply house act equally well.

The irregularity of the results obtained by us with inulin-serum-water medium has not been satisfactorily explained. Since the difference in favor of the bouillon might be referred to a greater quantitative development of bacteria in this medium, we have tested media with three actively-growing type cultures of the pneumococcus, varying in their fermentative activity, and with one culture of Streptococcus pyogenes. We have estimated by plating after twenty-four hours of growth the number of bacteria in plain bouillon, plain serum-water, dextrose-bouillon, dextrose-serum-water, inulin-bouillon, and inulin-serum-water. There was no difference in the number of colonies developing on the plates from the bouillon media and those from the serum-water media.

Our conclusions with regard to the inulin test are as follows:

I. The routine isolation of the pneumococcus is much facilitated by this test. Until the number and pathological significance of the non-inulin-fermenting group of pneumococci is established, the test must be carefully controlled by the older methods of identification.

II. A final opinion as to the best method of using the inulin cannot be offered. We believe, however, that the inulin-bouillon method by mere inspection is as easy to carry out and as accurate as the serum-water method, and that with careful titrations it is more delicate as it offers a means of subdividing the inulin-fermenting pneumococci into definite groups—a differentiation impossible by the latter.

STREPTOCOCCUS MUCOSUS.

In the course of the investigation we have twice encountered a Gram-staining, encapsulated diplococcus with characteristics which make it peculiarly interesting. We have identified it with the diplococcus described by Richardson, and by comparison

of cultures with the coccus described by Howard and Perkins. As we have noted some points in its morphology, cultural features, and pathogenicity which do not appear in their descriptions, we will describe the organism in some detail.

Source.—The first of our cultures of this micrococcus was isolated from the lung of a case of acute lobar pneumonia. The lung showed gray hepatization. Our description is based on the study of this culture. Recently a second organism which has the same cultural features has been obtained from the pus from a mastoid abscess.

Morphology.—Morphologically this micro-organism is an encapsulated, lanceolate diplococcus. It retains Gram's stain. Its capsule persists in cultures and is readily stained by any capsule method; it is frequently stained by the ordinary staining methods. Fig. 1, for example, shows a stained preparation made according to Gram from a culture on blood-agar. In the preparation, capsules were not noticed, but the photograph shows them faintly stained. Figs. 2, 3, and 4 are from preparations also stained by the method of Gram slightly modified. Smear preparations on clean slides are made from the exudate of an animal recently dead after inoculation with the culture. After drying in the air they are thoroughly fixed in the flame. While the slide is still hot enough to cause the stain to steam, the preparation is covered with the solution of gentian-violet, which is left on the slide for one or two minutes. Iodin is applied in the usual way, but decolorization with alcohol is not completed. The thin parts of the smear are completely washed out, the thicker parts are left stained. Counterstaining is omitted. Some fields will now be found in which there is a sharp differentiation of the cocci and in which the capsule as well has retained the blue. The results are inconstant and it may be necessary to make many preparations before a good one is obtained. The result when successful is a sharp picture in which the details of the capsule and its relation to the coccus are better shown than by other methods. By the study of these preparations, appearances which we have endeavored to show by photograph can be made out. Fig. 2 (*a*) shows a single coccus surrounded by a complete circular capsule.

Fig. 2 (*b*) shows a pair of small cocci with a poorly defined line of demarcation between them. On opposite sides of the capsule and in the line of the transverse division between the individuals of the pair are a slight thickening of the capsule and a thin linear projection toward the center of the pair. At *d* in Fig. 2 and at *c* in Fig. 3 these lateral thickenings have increased in breadth and inward extension. The dividing line between the cocci is better marked. At *e*, Fig. 4, the division between cocci is complete and the capsule surrounds both elements.

The capsules can also be stained by other methods. If Wright's modification of the Romanowsky stain be used, the cocci take a blue stain and the capsular portion takes the pink color. The outline of the capsule is distinct and the points that we have endeavored to illustrate are brought out with greater delicacy.

Thionin blue in saturated aqueous solution stains the cocci and capsule from an exudate rather lightly. The stain must be steamed for a few moments and is then washed off with water. The capsule is delicately outlined in purple and the details before described are seen. The preparations made by these later methods are less suitable for illustration than those by Gram's method.

The capsules stain by either of the methods of Hiss, but the details here described are rarely brought out by them, the capsule appearing filled with the stain rather than outlined by it.

The capsule of this micrococcus is always present when the organism is growing and moist. It can be readily demonstrated by any of the special methods of capsule staining and with almost equal readiness by methods which do not ordinarily demonstrate capsules on the pneumococcus and streptococcus. In its development and division the capsule follows closely the division and separation of the coccus. By the modified Romanowsky stain the basophilic coccus can be well differentiated from the oxyphilic capsule. The capsule of this diplococcus is evidently more highly developed and differentiated than that of the pneu-

mococcus. In these facts there is some evidence that the capsule of this micrococcus may be a part of the bacterial cell. Possibly it is the precursor of the cytoplasm of the more highly developed unicellular plants.

Cultural Features.—The micrococcus grows well with a profuse, very watery growth on the surface of 1 % glucose-agar, .5 % acid to phenolphthalein, if about 1 % of "fresh" defibrinated blood be added. The individual colonies are perfectly transparent and attain in forty-eight hours a size 6 mm. in diameter. It also grows in a glucose-agar stab culture without blood if the medium is made from beef and is suitable in reaction. In this way there can be developed peculiar and, so far as our experience goes, characteristic wing-like lateral out-growths. This feature was noted by Richardson, and together with the persistence of the capsules in culture serves to identify his culture with those that we have isolated. In gelatin-agar mixtures of proper reaction and sugar content growth is good, but lateral outgrowths in stab cultures do not appear. Abundant growth can be obtained on any of the usual solid or fluid media if .5 c.c. of fresh blood be added to each tube. The published data on the fermentation reactions are scanty. Hiss states that the organism of Howard and Perkins ferments inulin. We find that if fresh defibrinated blood be added to tubes of serum-water medium containing dextrose, lactose, maltose, galactose, mannit, dextrin, or inulin, these organisms coagulate the medium in from twenty-four to forty-eight hours. Controls of plain serum-water plus blood remain fluid. In litmus milk our organism produces very slight acidity after some days. In this respect it seems to differ slightly from those as described by others, which become markedly acid in twenty-four hours.

We have compared our organism with that of Howard and Perkins, obtained through the kindness of Dr. Libman, of Mt. Sinai Hospital, New York. In morphology and character of growth on blood-agar and in dextrose-agar they are identical. They react in the serum-water-sugar media in much the same way. Both coagulate the sugar-containing media before mentioned, but there are slight differences in the reaction time. To

compare the acid production we have added 0.5 c.c. of fresh sterile defibrinated horse's blood to tubes containing 10 c.c. of bouillon with 1 % of the various sugars. The results are shown in the following table:

	Acidity in Percentage of Normal Acid Solution.	
	Our Organism.	Organism of Howard and Perkins.
Bouillon plus blood	1.1 %	1.7 %
Bouillon plus blood with 1 % mannit	1.6 %	1.4 %
Bouillon plus blood with 1 % inulin	1.6 %	3.4 %
Bouillon plus blood with 1 % dextrose	4.0 %	4.0 %
Bouillon plus blood with 1 % lactose	3.9 %	3.8 %
Bouillon plus blood with 1 % maltose	4.0 %	3.8 %
Bouillon plus blood with 1 % galactose	3.9 %	4.0 %
Bouillon plus blood with 1 % saccharose	4.1 %	3.8 %
Bouillon plus blood with 1 % dextrin	4.0 %	3.3 %

Uninoculated control .9 %

The figures show that our organism differs from that of Howard and Perkins in the amount of acid produced in the presence of inulin under given conditions. Neither of them produces marked acidity in the mannit-blood-bouillon. Since the serum-water medium with the addition of blood and each of these sugars was coagulated, the greater delicacy of the titration method of demonstrating these reactions is again shown. When the total acidity produced by a culture under the conditions of the experiment rests at about 1.5 % normal acid, the reaction in the serum-water medium is apt to be uncertain. The identity of the reaction in serum-water with that in bouillon is again brought in question.

The hæmolytic activity of these two cultures has been roughly estimated in the following way: to tubes of plain beef bouillon .5 c.c. of defibrinated horse-blood are added. Tubes are inoculated and incubated with controls. In twenty-four hours the height to which the color ring of dissolved hæmoglobin rises in the

tube is noted. The hæmolysis is markedly greater with our organism than with that of Howard and Perkins. This seems to agree with the fact that when our culture was first isolated we had great difficulty in growing it except in the presence of blood. No such difficulty is noted by others who have isolated the organism. Efforts to obtain a hæmolysin or toxin that would pass a filter have so far been without result.

Pathogenicity.—The diplococcus with which we have worked is pathogenic for mice, guinea-pigs, and rabbits. When first isolated, one loop of blood-agar culture twenty-four hours old sufficed to kill a guinea-pig or young rabbit in twenty-four hours with general septicæmia and local exudation. At present several loops are necessary to bring about the same result. Smaller doses cause death in several days with fibrinous inflammations of the serous cavities. The exudate is characteristic; with some exceptions the amount of fibrin is small, but the total exudate is relatively large. It is white, and has the consistency of thick mucilage. If dropped from a pipette into salt solution, the drop holds together, sinks to the bottom, and remains intact for some time. There is little tendency to hæmorrhage either after subcutaneous or intraperitoneal inoculation. One old rabbit which survived a small dose developed after several weeks multiple arthritis with much exudate in the joint cavities.

Previous observers have considered this organism a streptococcus. The names Streptococcus capsulatus and Streptococcus mucosus have been applied to it. Morphologically it seems much more closely related to the pneumococcus. The lanceolate diplococcus form and arrangement are well marked, as shown by Fig. 1, and are well preserved under prolonged cultivation. Chains occur, but they are no larger than is usual with the pneumococcus. They seem to occur under much the same unfavorable conditions that tend to chain formation with the pneumococcus, and may possibly represent an involution. In its fermentation reactions it is very active and more closely resembles the pneumococcus than the streptococcus, although our culture differs from that of Howard and Perkins in this respect. Its pathological reactions also resemble the pneumococcus in that the organism appears in

the blood after injection of pure or mixed culture somewhat more readily than does the streptococcus. The organism is apparently a highly specialized pneumococcus.

DESCRIPTION OF PLATE.

Fig. 1.—Streptococcus mucosus. Stained by Gram's method and magnified 1000 diameters to show the morphology of the culture described.

Figs. 2, 3, 4.—Streptococcus mucosus. Stained by the modified Gram's method and magnified 1000 diameters to show capsules.

a. Single coccus with complete capsule.

b. Capsule shows slight lateral thickening and line projecting toward center.

c. Very heavy lateral thickenings in capsule.

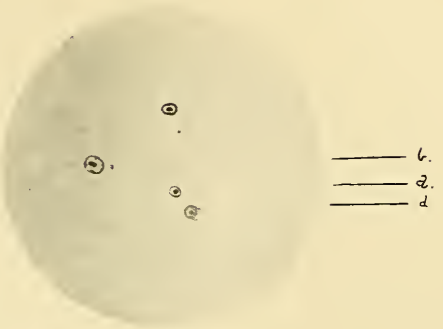
d. Somewhat heavier lateral thickenings in the capsule.

e. Capsule completely surrounding two cocci.

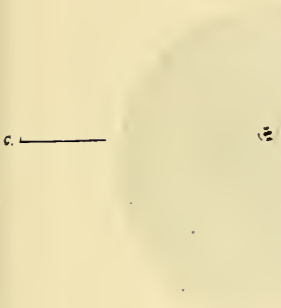
Fig. 5.—Pneumococcus from a culture on blood-agar twenty-four hours old. Stained by Hiss' copper-sulphate method for capsules and magnified 1000 diameters. No serum was added to the preparation.

Fig. 6.—Culture of pneumococcus (C. D. 30) on blood-agar twenty-four hours old (second generation); stained by Gram's method and magnified 1000 diameters. Showing the formation of chains composed of rather distinct lanceolate pairs.

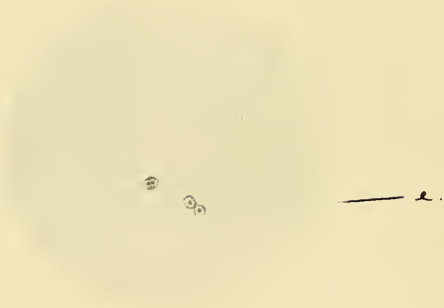
1.



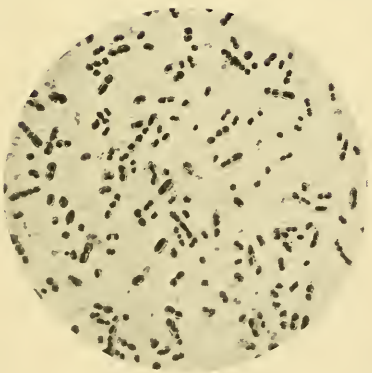
2.



3.



4.



5.

6.

STUDIES OF THE PNEUMOCOCCUS AND ALLIED ORGANISMS WITH REFERENCE TO THEIR OCCURRENCE IN THE HUMAN MOUTH.

By LEO BUERGER, M.D.

Assistant in the Pathological Laboratory, Mt. Sinai Hospital, New York.

The work upon which I shall report in this paper was carried on from November, 1904, to April, 1905, in the Laboratory of the Mount Sinai Hospital, at the request of the Commission for the Investigation of Acute Respiratory Diseases, of the New York City Board of Health.

The general scope is set forth under the following headings:

I.—The occurrence of the pneumococcus in the mouths of normal individuals.

II.—The evidence of communicability of the pneumococcus from one person to another.

III.—The study of the pneumococci isolated.

IV.—The study of organisms found in the mouths of normal individuals which closely resemble the pneumococcus.

V.—The identification of the pneumococcus; features of most diagnostic importance.

VI.—The agglutination of the pneumococcus and allied organisms.

VII.—General summary.

THE OCCURRENCE OF THE PNEUMOCOCCUS IN THE MOUTHS OF NORMAL INDIVIDUALS.

The studies on the occurrence of the pneumococcus in the mouths of normal individuals were conducted in two series.

1. By isolation of the pneumococcus from the mouths of ward patients, doctors, and nurses, not suffering from pneumonia.

2. By an examination of throat cultures on Loeffler's serum taken from individuals not suffering from inflammations with which the pneumococcus is usually associated, and by the morphological identification of the pneumococcus in such cultures.

The first series will be referred to as the "completely studied cases," the second as the "throat-culture series."

Completely Studied Cases.

Technique.—The material from which the isolations were made was obtained in the following manner. Sterilized swabs, such as are used for making throat cultures, were carried to the bedside, and the secretions of the mouth from the region of the posterior pharyngeal wall, pillars of the fauces, and tonsils were obtained by means of them. In a number of cases, additional material for cultures was obtained by collecting saliva in sterile test-tubes.

For the recognition and identification of the pneumococcus in the secretions, spreads of the secretion were stained, Loeffler serum cultures were examined, Petri plates were prepared, and in some instances animal inoculations were made.

Very little reliance was placed on the films. In most instances the number of organisms present in the original material was so small that they were not detected in the films, although the latter were stained both by the Gram and the capsule methods. At first thorough and painstaking search was made over these specimens. When it was subsequently found that the examinations were usually negative, even when the culture method proved the presence of the organism, less attention was paid to this procedure.

Loeffler serum tubes were inoculated directly from the swabs in every case. The tubes were incubated and spreads from various parts of the tube were stained after from eighteen to twenty-four hours' growth by the author's capsule method. It may suffice here to call attention to the fact that in very many, and in fact in the majority of, instances, the presence of pneumococci in the mouth can be diagnosticated by this simple procedure. By the employment of direct inoculations on Loeffler serum in every case, side by side with the plate method, I was able to determine the respective values of these two procedures. The full data will be given under the section dealing with the incidence of the pneumococcus in normal mouths.

The technique employed in the morphological identification of the organism on the Loeffler serum was the following: In most instances one or two tubes were inoculated directly by means of swabs, but a series of tubes, the inoculations being made on successive tubes until the swab was fairly dry, was also made in a number of cases.

The plan finally adopted was to examine the cultures after eighteen to twenty-four hours' growth, the spreads being made as follows: A number of loops from various parts of the tube was removed and thoroughly mixed with a few drops of a dilute beef-serum (beef-serum [1] and normal saline solution equal parts by volume) previously placed upon a clean slide. A loop of this mixture was carefully spread upon a perfectly clean cover-slip and stained by the author's capsule method.[2]

The characteristic appearance of the pneumococcus and its variations, when stained in the manner described, will be discussed in another section. The close resemblance of this organism and the small coccoid and diplococcoid forms of the Friedländer bacillus, so frequently met with in cultures from the human mouth, made it necessary to employ some differential method. The use of the capsule stain combined with the Gram stain made the differentiation of the two organisms in spreads possible. It was found that both the capsules of the pneumococcus and Friedländer bacillus take the counterstain, and the body of the former remains stained, whereas that of the latter is decolorized.

The examination of the Loeffler-serum cultures in the manner described was therefore conducted as a matter of routine in every case. It served as a fairly reliable indicator as to the presence of pneumococci in the plates. The value and reliability of a morphological identification of the organism was studied first, by comparing the results obtained in the plate isolations with the examination of the throat culture in every case, and secondly by plating out a large number of positive tubes and demonstrating the presence of the organism in pure culture.

In the development of a method which would be rapid and satisfactory for the isolation of the pneumococcus, a number of factors had to be taken into consideration. It was deemed important in dealing with an organism which is known to suffer such considerable alteration in morphology and virulence on culture media, to be able to obtain pure cultures which would be but little changed from the original type. The general aim was to observe the peculiarities of morphology, cultural characteristics, metabolic activity, and virulence of each strain.

The two methods which were adopted for the recovery of the organism were

---

[1] Undiluted rich pleural or ascitic fluid may be substituted.

[2] The method is as follows: Before the spread is completely dry it is covered with Zenker's fluid minus acetic acid and gently warmed for 3 to 5 seconds over a small flame. It is washed rapidly in water and flushed once or twice with 80 to 95 % alcohol, and then covered with tr. iodine which is allowed to remain on the cover-glass from 30 to 60 seconds or even longer. The iodine is washed off in alcohol and the specimen dried in the air. The staining is done with fresh aniline oil-gentian-violet in 2 to 5 seconds, the excess of stain being removed with 2 % salt solution. The preparation is examined in this fluid. See the *Medical News*, 1904, lxxxviii, 1117. Double staining can be carried out as by the capsule and Gram stains. After the specimen is dried in the air, it is stained by the usual Gram method, then counterstained with strong fuchsin (sat. alcoholic sol. 10 to 15 parts, water to 100 parts) for a minute or longer, washed in water and mounted in that fluid.

first plating and second inoculation of animals. The former is more tedious, the latter gives one a strain altered perhaps both in virulence and other properties. During the course of these experiments certain modifications and improvements were developed, which made me adhere to the plate method throughout the series of experiments to which I have referred.

My attention was directed first to the nature of the medium most suitable for plating, and second to the plating itself. Observations were made on the rapidity and luxuriance of growth of a number of pneumococci on serum-agar, and on serum-glucose-agar, made up with varying amounts of peptone, with or without the use of meat infusion, and of various titers. Profuse growths were regularly obtained on all these media.

It was decided to employ an agar or a 2 % glucose-agar of a *neutral*, or, at most, 0.5 % phenolphthalein acid titer. The medium was usually made from meat infusion and contained 1.5 to 2 % peptone and 2.5 % agar. Stock plates of these media (serum-agar and 2 % glucose-serum-agar) were poured. The agar or glucose-agar was melted in large tubes and allowed to cool down to a temperature below the coagulation point of the serum. One third volume of rich albuminous ascitic fluid was added, and the resulting medium poured into Petri-plates. These were tested by incubation and stored in the ice-chest, ready for use.

These plates were used for isolating the pneumococcus. At first the serum-agar and glucose-serum-agar were used side by side. It was found that although the former gave quite as luxuriant growths as the latter on slants, for the purposes of rapid isolation and detection of the organisms, the sugar medium, as pointed out by Libman,[3] was to be preferred.

Although in the serum-glucose-agar good growths are obtainable even when the reaction of the original agar is 1.2 % acid, it was deemed advisable to use as low a titer as would be compatible with good growth. Neutral glucose-agar, which upon the addition of ascitic fluid became slightly alkaline, afforded a medium which was very favorable for the growth of the pneumococcus. That the acid production at the end of eighteen to twenty-four hours did not raise the titer of such a medium to a very high point was shown by employing streptococci which were known to be abundant acid producers in glucose media. When cultivated on a more acid glucose-serum-agar (one made up from 0.8 % to 1.2 % acid glucose-agar), they made manifest the high acid titer by abundant precipitation or whitening after the stated period of time. When grown on alkaline agar, this was not apparent until almost double the time had elapsed.

The technique of plating required modification. By the use of the usual method, very many colonies are buried, and as deep pneumococcus colonies present nothing sufficiently definite to aid in their recognition they may be overlooked. When, besides this, the great variety of the flora in the human mouth and the usual numerical preponderance of other organisms over the pneumococcus are considered, it is clear that it is important to have a great number of surface colonies from which to pick. Both the usual streak method, and one carried out by wiping a loop over the whole surface of a plate, by a to-

---

[3] *Journal of Medical Research*, 1901, vi, 54.

and-fro lateral motion, proved unsuccessful. The plan finally adopted was as follows:

A swab taken from the mouth was thoroughly shaken in a tube of neutral bouillon. From this primary tube dilutions in bouillon with four, six, and eight loops may be made. A small portion of the dilute mixture was poured at a point near the periphery of the prepared plates. By a slight tilting motion the fluid was carefully distributed over the whole surface of the plates. Care must be taken to avoid an excess of fluid. It was found that plates made in this way gave a sufficiently thick and discrete distribution of surface colonies. As a rule, a number of plates were poured from the eight-loop mixture. These were generally satisfactory. In plating from growths on Loeffler serum the same plan was followed. One, two, and four loop-full mixtures were made from a primary tube containing approximately $\frac{1}{10}$th loop of the culture, and poured as above.

Films, Loeffler tubes, and plates were made from each case. Eighteen to twenty-four hours later the examinations of the cultures were made. As already stated, the spreads were usually negative, while the Loeffler cultures often showed the presence of pneumococci. The plates were also studied for the characteristic colonies.

The colonies of pneumococci are described rather indefinitely by most authors as being colorless, almost transparent, and watery. When studied on the media recommended, many strains present very characteristic colonies. The surface colonies of the pneumococcus take on the form of small flattened disc-like growths. When viewed from above, and by means of reflected light, the surface looks glassy, either flat or slightly depressed in the center. When looked at from the side, or more especially by transmitted light, the colonies appear as distinct rings, enclosing an almost transparent central area. When held up against the light, these rings look milky and somewhat opaque. The ring form is very characteristic for most of the pneumococci directly isolated from the mouth. It must not be understood that all pneumococci grow in this way, or that the same organism will do so under all conditions. At times a more mucoid colony without the ring shape is the type for some strains. However, a sufficiently large number of the organisms were found to show this form to warrant a careful search for them in the plates. Streptococci were found in rare instances to grow in ring-like colonies. These, however, when carefully examined were found to have a distinct nucleus, or more opaque center, thus differing from the transparent almost invisible center of the pneumococcus colonies.

It was customary to pick off a portion of the suspected colonies and stain it by means of the capsule method. When the typical "ring-form" was present, the typical encapsulated pneumococcus forms were generally found. When the colonies were too small, subcultures were directly made, and the morphological identification postponed a day. Positive colonies, spreads of which had shown encapsulated diplococci, were cultivated on tubes of serum-agar. From this point on, sugar media were avoided, and transplantations were regularly made on ascitic-serum-agar. Twenty-four hours later the surface growth was employed for tests of pathogenicity, and subcultures used for further morphological, cultural, and other tests. In this way it was possible

in most instances to inoculate test animals with pure cultures of the organism forty-eight hours after the plates were poured, and with the second generation of the organism.

White mice were employed for tests of virulence, the heightening of the virulence of certain strains, for testing doubtful organisms, and for direct inoculations of saliva.

In testing virulence, inoculation, whenever possible, was made with the second generation of the pneumococci. The surface growth of a 24-hour culture on serum-agar was suspended in 1 c.c. of normal saline solution and injected subcutaneously. These animals served not only as a means for determining the pathogenicity of the organisms, but also to prove the correctness of the diagnosis by presenting the typical encapsulated forms in the exudate and blood.

A heightening of virulence was resorted to for the purpose of obtaining strains which were to be used for the immunization of rabbits. Tests to increase the virulence of organisms resembling the pneumococcus, and of avirulent forms of the pneumococcus were also made.

Doubtful organisms, particularly those in which typical capsule forms were not demonstrable in culture, were also put into mice for purposes of diagnosis.

CONSIDERATION OF THE CASES.

In this place will be given the results of the studies on the occurrence of the pneumococcus in normal mouths. Although the mouths were not always strictly normal, those which for all intents and purposes could be regarded as such, fell within the scope of the work. In some cases there was slight hyperæmia, moderate catarrhal inflammation, or slight follicular hyperplasia. Notes on these conditions were always made.

The material for study was derived in the main from the hospital wards. The total number of normal cases studied was 78. Cultures were also taken from doctors, nurses on duty, and a few from private persons outside the hospital. Some of the diagnoses in the sick were multiple neuritis, brain tumor, rheumatism, osteomyelitis, appendicitis, nephritis, and cirrhosis of the liver. In a general way it may be said that the work was planned with a view of obtaining evidence of communicability from person to person of the pneumococcus. Negative cases were examined two, three, or more times in the attempt to discover the organism, and positive cases were also re-examined. In the latter, data regarding the persistence of pneumococci in the mouth were

sought. The 78 persons studied consisted of 49 adult males, 24 adult females, and 5 children.

The percentage of those in which pneumococci were found at some time or other was fifty.

The following tabulation gives the percentage of positive results in the case of adult males, females, and children.

	Total Number.	Positive.	Percentage.
Adult Males	49	25	51.0
Adult Females	24	11	45.8
Children	5	3	60.0

In the above so-called "normal cases" there was one child in whom diphtheria bacilli were isolated from the throat.

In addition to the normal cases, the presence of pneumococci was determined also in fifteen cases of pneumonia. Most of these were cases of frank lobar pneumonia, others were cases of broncho-pneumonia, one was a case of grippe-pneumonia, while in one case only was the Friedländer bacillus found. These pathological cases were added to the list for the following reasons: first in order to obtain information regarding the reliability of the cultural methods employed; second as controls in the studies made upon the communicability of the pneumococcus from the mouths of pneumonia cases to other patients in the ward; and third for a comparative study of the organisms isolated from them and from normal persons.

Of the total fifteen cases there was one case as stated in which the Friedländer bacillus but not the pneumococcus was isolated, and another case of "influenza pneumonia" in which neither pneumococcus nor the pneumo-bacillus could be discovered. In the remaining thirteen cases the Diplococcus lanceolatus was demonstrated once or more times. Twelve strains were isolated and studied. By repeated examinations of the cases, information regarding the persistence of the organism in the mouths of such patients was obtained.

Before entering upon a more detailed study of the normal and

pneumonia cases, it may be well to append a table (I) giving the condition of mouth and pharynx in each patient.

TABLE I.

CONDITION OF THE MOUTH AND PHARYNX.

Case No.		Case No.		Case No.	
1.	Follicular hypertrophy.	32.	Negative.	63.	Negative.
2.	Negative.	33.	"	64.	" (pneumonia).
3.	" (pneumonia).	34.	" (pneumonia).	65.	" "
4.	Diphtheria.	35.	Negative.	66.	" "
5.	Large left tonsil.	36.	Slight pharyngitis.	67.	Negative.
6.	Negative.	37.	Neg. (pneumonia).	68.	" (pneumonia).
7.	"	38.	Negative.	69.	Negative.
8.	"	39.	Slight congestion.	70.	"
9.	"	40.	Negative.	71.	"
10.	Slight pharyngitis.	41.	"	72.	"
11.	Negative.	42.	"	73.	"
12.	Slight tonsillitis.	43.	"	74.	"
13.	Hyperæmia.	44.	"	75.	"
14.	"	45.	" (pneumonia).	76.	"
15.	Negative.	46.	Slight congestion.	77.	"
16.	"	47.	Negative.	78.	"
17.	"	48.	Slight congestion.	79.	"
18.	"	49.	Negative.	80.	Congestion.
19.	" (pneumonia).	50.	Slight congestion.	81.	"
20.	Negative.	51.	Negative.	82.	Occasional congest.
21.	"	52.	"	83.	Large tonsils.
22.	"	53.	"	84.	Congested ?
23.	"	54.	Large phar. follicles.	85.	Slight congestion.
24.	"	55.	Negative.	86.	Negative.
25.	" (pneumonia).	56.	"	87.	"
26.	Negative.	57.	"	88.	Congestion.
27.	"	58.	"	89.	"
28.	" (pneumonia).	59.	"	90.	Negative.
29.	Negative.	60.	" (pneumonia).	91.	"
30.	"	61.	" "	92.	Congestion.
31.	" (pneumonia).	62.	" "	93.	Negative.

A survey of this table will show that, apart from slight congestion (which was not present at every examination) or follicular hyperplasia, most of the throats examined were negative for pathological conditions. The cases are given in the order of their study.

In order to facilitate future reference, both as to the organisms isolated and the cases themselves, I have tabulated the completely studied cases, indicating whether they were normal or pneumonia cases, and also noting the number of the cultures of pneumococcus or allied organisms. Whenever more than one culture was isolated for thorough study, more than one number will appear in the respective column.

TABLE II.

CASES AND CULTURES.

Case.	Name.		Pneumococcus**	Not Studied.†	Other Organisms.‡
1.	H. S.	N*	—	—	—
2.	J. F.	N	—	+	—
3.	S. R.	P	E 1	—	—
4.	A. L.	N	E 2	—	—
5.	S. S.	N	—	—	—
6.	A. B.	N	—	—	—
7.	M. F.	N	—	—	—
8.	G. S.	N	—	—	—
9.	J. E.	N	—	—	—
10.	L. B.	N	—	—	—
11.	I. H.	N	E 3	—	—
12.	Private E. L.	N	E 4	—	—
13.	A. H.	N	E 5	—	—
14.	Private E. Z.	N	E 6	—	—
15.	J. H.	N	—	—	—
16.	S. K.	N	—	—	—
17.	Nurse S.	N	—	—	—
18.	S. G.	N	—	—	—
19.	S. S.	P	E 7	—	—
20.	B. R.	N	—	—	—
21.	A. St.	N	—	—	—
22.	Nurse G.	N	—	—	E 31
23.	Nurse M.	N	—	+	—
24.	S. Sh.	N	E 16, 19	—	—
25.	A. Se.	P	—	—	F
26.	Al. B.	N	—	—	—
27.	Nurse W.	N	—	—	—
28.	L. G.	P	E 8	—	—
29.	W. B.	N	E 9	—	—
30.	J. M.	N	—	+	—
31.	H. C.	P	E 10	—	—
32.	S. St.	N	E 11	—	—
33.	N. G.	N	E 12	—	—
34.	M. S.	P	E 13	—	—
35.	H. K.	N	E 14	—	—
36.	N. S.	N	E 15, 20, 22	—	—
37.	T. C.	P	—	+	—
38.	J. B.	N	—	+	E 42
39.	H. P.	N	E 18	—	—
40.	E. W.	N	—	—	F
41.	A. B.	N	E 17	—	—
42.	S. W.	N	E 24	—	—

* N means "normal"; P, pneumonia; F, Friedländer bacillus infection.

** Cultures in this column were identified as pneumococci and will be designated by the letter E followed by a number. They were isolated from the case occupying the same line.

† The plus mark indicates that in these cases pneumococci were found but not completely studied.

‡ Other organisms, i. e., doubtful strains, unidentified forms, and Streptococcus mucosus capsulatus are designated by letter E or otherwise.

TABLE II (*Continued*).

Case.	Name.		Pneumococcus.	Not Studied.	Other Organisms.
43.	M. S.	N	E 21	—	—
44.	M. St.	N	E 48	+§	—
45.	B. R.	P	E 23	—	—
46.	B. S.	N	E 40	+	—
47.	S. C.	N	—	—	—
48.	I. P.	N	—	—	—
49.	M. So.	N	—	—	—
50.	M. G.	N	—	—	F
51.	R. Kr.	N	E 25	—	—
52.	A. Be.	N	—	—	—
53.	H. W.	N	E 26	—	—
54.	H. Ka.	N	E 27	—	+§
55.	A. Kr.	N	E 39	—	E 30
56.	T. S.	N	—	—	—
57.	N. M.	N	E 28, 29	—	—
58.	A. S.	N	—	+	—
59.	M. W.	N	—	—	—
60.	D. B.	P	E 32	—	—
61.	F. T.	P	E 33, 36, 37, 38	—	—
62.	M. G. ‖	P	—	—	—
63.	S. K.	N	E 45	—	E 41
64.	R. M.	P	—	+	—
65.	A. S.	P	E 35	—	—
66.	M. Bo.	P	E 34	—	—
67.	I. K.	N	—	—	—
68.	M. Rei.	P	E 43	—	—
69.	F. C.	N	E 47, 54	—	—
70.	A. C.	N	E 44, 46	—	—
71.	F. R.	N	—	+	—
72.	L. G.	N	—	+	—
73.	R. E.	N	—	—	—
74.	D. G.	N	—	—	—
75.	S. F.	N	—	—	—
76.	H. G.	N	E 49	—	—
77.	E. M.	N	—	—	—
78.	K. J.	N	—	—	—
79.	L. W.	N	—	—	—
80.	A. N.	N	E 50	—	—
81.	H. C.	N	E 51	—	—
82.	M. Fe.	N	—	—	—
83.	I. B.	N	—	—	—
84.	E. A.	N	—	—	—
85.	A. Bo.	N	E 52, 56	—	—
86.	C. W.	N	E 53	—	—
87.	J. J.	N	—	—	—
88.	B. St.	N	—	—	—
89.	N. H.	N	—	—	—
90.	L. W.	N	—	—	—
91.	B. H.	N	—	+	E 57
92.	M. St.	N	—	—	—
93.	B. Ko.	N	—	E 55	S. M. ¶

§ In these cases at one time the organism was identified but not studied; at another time culture marked E was isolated.

‖ Influenza pneumonia case. ¶ Streptococcus mucosus.

The Persistence of the Pneumococcus in the Mouths of Normal Persons.—In the study of the organism in the so-called normal cases, the repeated occurrence of the pneumococcus in the same individual was often demonstrated. Time did not permit of the complete study of all the organisms isolated in this way. Whenever the persons themselves or the particular strain of organisms presented points of unusual interest, a number of different strains was isolated and studied. Reference to the foregoing table will show that in some patients, two, three, or four pneumococci (marked E) obtained on different occasions were identified and kept for study. In many of the other cases, however, when cultures were repeatedly positive the fact alone was recorded, and the organism itself merely identified.

For the purpose of convenience the results of the observations on a number of the cases in which the pneumococcus was found more than once are tabulated. A number of instances in which there were several negative cultures and one positive one is also included. The data may be taken as showing that the pneumococcus is to be found in normal human mouths for days, weeks, or even longer.

In the following table the words "positive" and "negative" [4] indicate whether or not pneumococci were cultivated from the mouth on the corresponding dates:

TABLE III.

PNEUMOCOCCUS IN THE NORMAL MOUTH.

Case 2.

Nov. 25, negative.
" 27, positive.
Dec. 4, negative.
" 11, "
" 20, "
" 28, "

Case 13.

Dec. 3, positive.
" 9, "
" 18, "

Case 24.

Dec. 4, negative.
" 10, "
" 16, positive.
" 19, negative.
" 20, positive.
" 23, negative.
" 27, positive.
" 28, "
Jan. 1, negative.
" 3, positive.
" 4, "
" 6, negative.

Case 29.

Dec. 7, negative.
" 18, positive.
" 26, negative.
" 31, "
Jan. 20, "

Case 30.

Dec. 20, negative.
" 23, positive.
" 27, negative.

[4] The phrases "positive case" and "negative case" are used throughout this paper to indicate the presence or absence of pneumococci in the mouth.

TABLE III (*Continued*).

Case 32.

Dec. 20, positive.
" 21, "
" 26, "
" 31, negative.

Case 35.

Dec. 20, positive.
" 23, "
" 24, "

Case 36.

Dec. 27, positive.
" 28, "
Jan. 1, "
" 6, "
" 7, "
" 8, "
" 9, "

Case 39.

Dec. 28, positive.
" 29, "
" 30, "
Jan. 3, negative.
" 16, positive.
" 19, "
" 29, negative.

Case 42

Dec. 27, negative.
Jan. 4, positive.
" 8, negative.
" 9, positive.
" 11, negative.
" 15, "
" 18, positive.
" 24, negative.

Case 51.

Jan. 11, negative.
" 14, positive.
" 16, "
" 18, negative.
" 24, "
Feb. 7, "

Case 54.

Jan. 14, positive.
" 15, "
" 16, "

Case 55.

Jan. 11, negative.
" 15, "
" 16, "
" 19, "
" 26, positive.
" 27, "
" 29, negative.

Case 57.

Jan. 19, positive.
" 20, "
" 21, "
Feb. 5, negative.

Case 63.

Jan. 24, negative.
" 26, "
" 27, "
" 29, positive.
Feb. 7, negative.

Case 69.

Feb. 2, positive.
" 13, "
" 28, "

Case 70.

Feb. 3, positive.
" 7, "

Case 85.

Feb. 23, positive.
Mar. 2, "

Before analyzing this table I wish to present the results of the examinations in another form. In this the longest time which elapsed between two positive cultures in the same case will be indicated, and the total number of examinations made in each instance and the total number of positive findings recorded. All the cases in the last table are not included in Table IV.

TABLE IV.

Case.	Time.*	Examinations.†	No. Positive.‡
13	15	3	3
24	19	11	6
32	6	4	3
35	4	3	3
36	12	6	6
39	22	7	5
42	14	8	3
69	26	3	3
70	4	2	2
85	10	2	2
Total 10	—	49	36

* The number of days which elapsed between the first and last examination.
† The number of examinations made on different days.
‡ The number of times pneumococci were found.
Cases in which only one positive culture was obtained and those in which the time between two positive examinations was only one or two days are omitted.

Examination of Tables III and IV brings out some interesting points. In the first place it is seen that one or more negative examinations often preceded the positive finding. A single set of negative cultures is of course not convincing. In some cases, however, the absence of the organism was noted on two, three, or more days before the first positive find. Such cases are certainly to be regarded as furnishing evidence of the fact that the pneumococcus may suddenly appear in normal mouths which have been previously shown to be free from them. The following are some such instances:

Case 2, negative, Nov. 25;	positive, Nov. 27.
" 55, negative, Jan. 11, 15, 16, 19;	positive, Jan. 26.
" 63, negative, Jan. 24, 26, 27;	positive, Jan. 29.

Another fact to be mentioned is the regularity with which the pneumococcus was found in certain persons. Thus it was present:

In Case 13,	3 times	in	3 examinations.	
" " 35,	3 "	"	3	"
" " 36,	6 "	"	6	"
" " 69,	3 "	"	3	"

In the latter, the intervals between the first and last examination were 15, 4, 12, and 26 days, respectively. These patients of this table left the hospital shortly after the last culture was taken and hence could not be studied further.

The second column of Table IV shows how long pneumococci were actually found to exist in some of the cases. The shortest time is four days, the longest twenty-six. There seems little doubt that these figures might have been larger had the observations been extended over a longer period of time.

There is another series of cases, of which numbers 24, 39, and 42 are examples (Table III), which deserves mention. In these the organism was not detected at every examination, but very frequently. That the bacterial flora of the mouth must undergo changes throughout the day is highly probable. The mechanical and chemical influences to which the mucous membrane is subject are necessarily great. It is very probable, then, that

repeated examinations made on the same day might have revealed the organism where a single culture proved negative.

From a consideration of the foregoing, there would seem to be evidence that the pneumococcus may suddenly appear in the so-called normal mouth, that certain individuals carry the organism in their mouths for considerable periods of time, and that even those persons in whom the organism cannot be demonstrated at every examination may harbor the pneumococcus in the less accessible portions of the respiratory tract.

THE STUDY OF CASES OF PNEUMONIA.

From this class of patients, as already mentioned, the pneumococcus was isolated as a control in the study of the communicability of the pneumococcus to other patients in the hospital wards. These cases also furnished proof of the reliability of the cultural methods employed and materials for use in the comparative study of the organisms from normal and pathological cases.

TABLE V.

PNEUMONIA CASES.

Case.	Pneumonia.	Pneumococcus.
3, S. R.	Lobar	positive.
19, J. S.	"	"
25, A. S.*	"	negative.
28, L. G.	"	positive.
31, H. C.	"	"
34, M. S.	"	"
37, T. C.	"	"
45, B. R.	"	"
60, D. B.	Broncho-	"
61, F. T.	Lobar	"
62, M. G.	Influenza	negative.
64, R. M.	Lobar	positive.
65, A. S.	"	"
66, M. B.	Broncho-	"
68, M. R.	Lobar	"

With the exception of Nos. 25 and 62, pneumococci were found in all. The former was a case of lobar and the latter a case of influenzal pneumonia. There were in all 12 lobar and two broncho-pneumonic cases.

* The Friedländer bacillus was isolated repeatedly; pneumococcus not found.

TABLE VI.

REPEATED EXAMINATIONS IN CASES OF PNEUMONIA.

Case 3.

Nov. 22,	positive (7th day) *	
Dec. 1,	"	—
" 5,	negative	—

Case 19.

Dec. 3,	positive (5th day)	
" 8,	"	—
" 17,	"	—
" 21,	negative	—
" 26,	positive	—
" 31,	negative	—

Case 28.

Dec. 15,	positive (11th day)	
" 17,	"	—
" 21,	"	—
" 23,	negative	—
" 26,	positive	—
" 31,	negative	—

Case 31.

Dec. 18,	positive (7th day)	
" 27,	"	—
Jan. 3,	"	—

Case 61.

Jan. 21,	positive (?)	
" 22,	"	—
" 23,	"	—

* Day of the disease. Whenever this column is found blank, the examination was made after resolution had set in or the crisis had occurred.

There was no difficulty in isolating the organism from these patients. In most instances several successive positive cultures were secured. No complete investigation of the length of time the pneumococcus could remain in the mouths of the patients was made. Such data as were obtained showed that they were present for many days after the crisis or after the beginning of resolution.

In Case 3, it was found 9 days after the crisis.[5]

" " 28, " " " 11 " " " "

" " 19, " " " 23 " " " "

" " 31, " " " 16 " " " "

Although the last recorded culture taken in Cases 3, 19, and 28 were negative (Table VI), it is possible that observations extending over a longer period of time may have given positive results.

PNEUMOCOCCI IN A SERIES OF CULTURES FROM THE THROAT.

It is intended in this section to give the results of studies made upon a separate series of cases. The plan of this part of the

[5] Beginning resolution or decided drop of temperature towards normal.

work was to obtain data regarding the incidence of the pneumococcus in a large number of normal cases, to ascertain the value of the morphological identification of the pneumococcus as compared with the other methods, and to determine in how far this last procedure would fall behind the plate method as a means for detecting the organism in the mouth.

Two hundred and four normal cases, not including the seventy-eight "completely studied" cases, were examined. In this series there were 71 positive and 133 negative cases, or 34.8 % of positive cases, being 15.2 % less than the percentage which was found in the "completely studied" cases. In order to explain this discrepancy it may be well to analyze the factors which may be responsible for the difference: 1st, the cases themselves; 2d, the imperfection of the method, and 3d, the morphological identification of the pneumococcus in Loeffler cultures. The difference in percentage may after all fall within the limits of this larger series of cases. But the fact that the examinations were made only once in each case could also account for the disparity.

The cultures were often taken on one tube only. A possible overgrowth by other organisms must be considered. This is to be avoided in many instances by the inoculation of several tubes in series with the same swab. Although in the preparation of films from the tubes care was taken to include many different portions of the surface growth, and although a number of specimens were examined in each instance, it is likely that a certain number of pneumococci may have been missed. Finally the morphological identification of the pneumococcus may be at fault.

In the spreads stained by the capsule method, the pneumococcus must be differentiated from a number of other encapsulated organisms: from the encapsulated streptococcus, the Streptococcus mucosus capsulatus, the diplococcoid forms of the Friedländer bacillus, the diplococcus forms of the Micrococcus tetragenus, the double cocco-bacillary organism frequently found in beef-serum, and from a large number of peculiar encapsulated diplococci and streptococci.

In 145 of the 204 throat examinations, notes were made in regard to the preponderating organism. In the following table the frequency of occurrence of the organisms appearing in greatest numbers is given for the 145 cases.

TABLE VII.

PREPONDERATING ORGANISMS IN THROAT CULTURES.

Streptococci	63
Staphylococci	42
Bacilli (not identified)	23
Friedländer bacilli	17

In this series the Friedländer bacillus occurred twenty-one times, or in about 14.5 %, and the Streptococcus mucosus capsulatus eight times, or in about 5.5 %.

In many of the cultures the mixture of organisms was so great that no single form could be estimated accurately. Very often the streptococci and staphylococci seemed to be present in equally large numbers. Only such bacilli were identified as belonging to the Friedländer group as exhibited typical capsules when grown on Loeffler's medium, as well as the characteristic mucoid growth. There was an exception in the case of an organism which was proven to belong to this class by isolation and subculture, but on which capsules were not demonstrated. Many of the bacilli were isolated and studied. The results of this part of the study furnish, therefore, further evidence of the frequent occurrence of the pneumococcus in the mouths of normal individuals.

STUDIES ON THE COMMUNICABILITY OF THE PNEUMOCOCCUS.

This part of our study was approached in two ways: by means of the histories of the individuals studied, and by repeated observations on the occurrence of the pneumococcus in the mouths of patients in the hospital wards.

Very little information in regard to the source of the pneumococci to be found in the positive cases could be obtained from the histories. The ignorance of the patients, their failure to remember the condition of those with whom they came into contact, and their inability, in most instances, to give reliable answers in regard to their association with pneumonia cases made obtaining any

reliable data unsatisfactory. In a very few cases there was a history of pneumonia in some member of the family, or in the individual himself. One positive so-called normal case had lived in the same house with a patient who was suffering from lobar pneumonia, and who was subsequently treated in the same ward.

Evidences of communication of pneumococci from one person to another were sought: by demonstrating the absence of pneumococci in the mouths of normal cases, by noting the appearance of the organism in the mouths of such cases, by studying the possible sources of infection and groups of patients who came in contact either with pneumonia cases or with persons in whose mouths the pneumococcus had been found, and by collecting other data in regard to the occurrence of the organism elsewhere in the hospital wards.

For this study two male wards, one containing twenty-four and the other twelve beds, and a medical children's ward were selected. The number of positive cases present in the small ward at any given time was rapidly ascertained; three successive days sufficed for taking cultures from all twelve cases, four being taken at a time. It was possible by the employment of the plate method to arrive at fairly definite conclusions after twenty-four to forty-eight hours. This process was repeated a number of times and the subsequent appearance of the organism in negative cases determined. The larger male ward and children's ward were studied in a similar manner, five or six cultures being taken a day.

Negative cases were repeatedly examined. Some of them could be followed from the day of their arrival in the wards. Others, such as cases of chronic nephritis, cirrhosis of the liver, chronic rheumatism, etc., had already been occupants of beds for days and weeks before the work was begun. In all of these it was possible to watch for subsequent development of pneumococci in the mouth.

The group of cases already referred to was selected for the purpose of observing the effect of the association of "negative" individuals, both with pneumonia cases and with those "normal" persons in whom large numbers of pneumococci were

repeatedly found. In certain circumstances, upon the arrival of a pneumonia patient in the ward the secretions from the mouth, as well as from the mouths of patients in the adjoining beds, were examined. These observations were repeated on a number of days. The same plan was adopted for the study of some of the normal "positive" cases.

In regard to other sources of infection, a number of handkerchiefs and cups belonging to "positive" cases were examined for the presence of pneumococci.

The following tables will give the results of several series of cases studied in wards, either in groups or singly. Table No. VIII refers to one of the children's wards. Isolated observations were made on these patients during a period of about one month and a half.

TABLE VIII.

OBSERVATIONS ON COMMUNICABILITY.

Bed.	Case.	Nov. 22	Dec. 4.	6–10.†	17–26	Jan. 1.	2–10.	3–4.	5–10.
1	normal *	neg.	neg.	—	neg.	pos.	pos.	neg.	neg.
2	"	"	"	—	"	neg.	—	—	—
3	"	"	"	—	"	"	—	—	—
5	pneumonia	pos.	—	pos.	pos.	"	—	—	—
6	normal	—	—	neg.	neg.	"	—	neg.	—
7	pneumonia	pos.	—	pos.	"	—	—	pos.	neg.
9	"	—	—	—	pos.	—	—	—	"
10	normal	—	—	neg.	neg.	—	neg.	—	—
11	"	—	—	"	—	—	—	—	—
12	"	pos.	pos.	"	pos.	—	neg.	—	—
13	"	—	—	pos.	—	—	—	neg.	—
Nurse 1	"	neg.	neg.	—	neg.	—	—	—	—
" 2	"	—	pos.	pos.	"				

* Normal, i. e., suffering from some disease which could bear no relation to the presence of pneumococci in the mouth.

† The examinations were made within the two dates. A dash signifies that no culture was taken; usually because the patient had left the ward or had not as yet been admitted.

In the first column of the table will be found the bed numbers of the cases under observation. There were eleven cases in all. Besides these, a night and day nurse (Nos. 1 and 2) are included in the list. On November 22d there were three patients with pneumococci in their mouths; two of these were cases of

pneumonia and one a "normal" case. On the 4th of December Nurse No. 2 was positive, but inasmuch as she had not been examined before, no deduction can be made. On the 17th of December, No. 9, another case of pneumonia, was admitted to the ward. Bed No. 1 now furnished an instance of possible acquisition of the organism by reason of association with positive cases. A girl with chorea was up and about the greater part of the day and frequently attended to the wants of certain other cases in the ward. Three examinations made between November 22d and December 26th gave negative results. On the 1st and 2d of January, however, the organism was found present in her mouth.

As an example of a case in which the appearance of pneumococci could possibly be traced to the patient in the adjoining bed, I may cite the following: Case 24 (Table II), a "normal" patient in Bed 1, was examined on the 4th and 10th of December and found negative. On the 10th of December a case of lobar pneumonia was put in the next bed. Pneumococci were repeatedly demonstrated in the latter patient, and on December 15th and on a number of days afterwards the same organism was detected in the "normal" patient, as is shown in Table IX.

TABLE IX.

Bed 1.	Bed 2.	Bed 4.	Bed 1.	Bed 2.	Bed. 4.
Dec. 4, neg.*	—	—	Dec. 23, neg.	—	—
" 10, "	pos.	—	" 27,† pos.	—	pos.
" 15, pos.	pos.	—	" 28, "	—	—
" 16, pos.	"	—	Jan. 1, neg.	—	—
" 19, neg.	—	Dec. 18, pos.	" 3, pos.	—	pos.
" 20, pos.	—	"	" neg.	—	—
" 21 "	—	—			

In the above table the dates and results of the various examinations are given. Another case of lobar pneumonia (Bed 4) was admitted on December 17th, and was regularly positive. The same ward furnished cases in which pneumococci were only occasionally found. The source of the organisms could certainly be referred to the large number of patients, pneumonia or normal, who habitually carried them in their mouths. Thus Bed 8, in Table No. 10, could possibly be regarded as having been repeatedly infected.

* Negative examinations were repeated in most instances.

† Moved into Bed 2 on this day.

A further series of similar observations now follow in tabulated form:

TABLE X.

OBSERVATIONS ON BED 8.

Bed 8.	Positive Cases.	Total No.*
Dec. 18, neg.	Beds 1, 2, 4, 9	4
" 28, pos.	1, 2, 3, 4, 10	5
" 29, pos.	1, 2, 3, 4, 10	5
" 30, neg.	1, 2, 3, 4, 10	5
Jan. 4, pos.	1, 2, 3, 4, 5, 10	6
" 10, neg.	1,† 2, 3, 4, 5, 6	6
" 15, pos.	—	
" 18, pos.	5	1

* Of other positive cases in the ward.
† A new case in the same bed, also positive.

The first column shows the occasional absence and appearance of pneumococci in the mouth of the patient in Bed 8. In the second column have been indicated the "positive" cases in the ward on the corresponding dates.

A similar series of notes was made in regard to Bed 5 of the same ward. The patient was a "normal" case. Three of the cases in adjoining beds had pneumococci in their mouths. The case in question probably acquired them in the ward.

TABLE XI.

OBSERVATION ON BED 5.

Bed 5.	Positive Cases.	Total No.
Dec. 27, neg.	Beds 1, 2, 4, 10	4
Jan. 4, pos.	1, 2, 4, 8, 10	5
" 8, neg.	—*	
" 9, pos.	—	
" 10, pos.	1, 2, 3, 4, 6	5
" 11, neg.	—	
" 15, neg.	8—	
" 18, pos.	—	

* Blank means that there was no record of the other ward cases on this day or on the days immediately preceding or following.

In addition similar observations on two other patients may be cited. The patient in Bed 6 was repeatedly negative for almost

a week after admission to the ward. At that time patients in Beds 1, 2, 4, and 10 had been regularly positive, the two last being cases of pneumonia. Cases in Beds 3, 5, and 8 were occasionally positive. On January 9th pneumococci were also found in case Bed 6. Two days later they had disappeared.

The second case was in Bed 3. He was admitted about the same time as the above case and was free from pneumococci. To his left were Beds 1 and 2, occupied by regularly positive cases, and to his right a convalescing pneumonia patient. Nine days after admission the organism was detected in the mouth of this patient.

The next study was carried out on a "normal" patient who was regularly positive, and consisted in the examination of the following parts and articles: Culture from his dry lips, handkerchief No. 1, handkerchief No. 2, sputum cup, culture from the lip of his drinking-cup.

The usual mouth cultures had given positive results on December 27, 28, January 1, 6, 7, 8, and 9th (Case 36, Tables II and III). E 15 was isolated and studied.

On January 7th a culture from the dry lips showed typical pneumococci. The patient's handkerchief, after twenty-four hours' use (and when perfectly dry), was examined for the presence of pneumococci. Numerous colonies of this organism were obtained. Culture E 20 was isolated. The handkerchief was kept in a sterile jar and examined thirty-six hours, and seven days later, and pneumococci were cultivated on these dates. On January 10th a second handkerchief was examined with positive results. A sputum cup which was filled with 5 % carbolic-acid solution, on the surface of which some of the patient's saliva was floating, was examined on January 7th. Cultures from the saliva showed typical pneumococci. A successful attempt was made to discover the organism on the patient's dishes. Cultures taken from the lip of a drinking-cup some five minutes after he had partaken of cocoa gave pneumococci (E 22).

Of the observations made on the larger male ward two instances only will be given, relating to two patients who had been

negative for some time before the pneumococci finally appeared. In the case of the patient in Bed 19, fifteen days elapsed before the organism appeared; in the case of the one in Bed 20, a full month.[6] Both of these patients in all probability acquired the pneumococcus from other cases in the same ward.

TABLE XII.

OBSERVATION ON BEDS 19 AND 20.

Bed. 19.	Bed 20.
Jan. 11, neg.	Dec. 27, neg.
" 15, "	Jan. 11, "
" 16, "	" 16, "
" 19, "	" 27, pos.
" 26, pos.	
" 27, "	
" 29, neg.	

The conclusions in regard to communicability which I have reached are:

(1) "Normal" individuals in whose mouths the pneumococcus is repeatedly found to be absent may acquire the organism by association with cases of pneumonia or with "positive normal" persons; (2) the handkerchiefs and dishes of pneumonia and "positive normal" cases may be regarded as means of transportation of the pneumococcus from person to person.

STUDY OF THE PNEUMOCOCCI ISOLATED.

The characters of the pneumococcus having already received so much attention at the hands of previous workers, much of the following study will necessarily be repetition. A point of especial importance was the relationship of the pneumococcus in the normal mouth to the organism obtained from pneumonia cases and from other pathological processes. In the study of the pneumococcus in the normal mouth and in the mouths of pneumonia cases, I have considered the typical characteristics and the variations which occur among a large number of different strains of pneumococci. I observed the morphology, cultural properties,

[6] It is of course recognized that positive results might have been obtained earlier by taking cultures at shorter intervals.

metabolic activity, pathogenicity, and agglutinative action of the organisms, studied comparatively the organisms from the two sources, and the best means of isolating the pneumococci, and considered what should be the determining factors in their identification.

Table II shows the sources of the organisms studied in this place, but in addition ten other strains, nine of which were isolated from cases of empyæma and one from a suppurating joint, were used by way of control in the study of morphology and cultural properties.

Morphology of the pneumococci.—The changes in form, which very many strains undergo on the various media, in exudates and blood of animals, and on the same medium from generation to generation may be very great. It is therefore of importance in a comparative study to observe the organisms under conditions which shall be as nearly alike in each instance as possible. In most cases the growths on Loeffler serum, and the colonies on the plates were examined with the capsule method from eighteen to twenty-four hours after the cultures were taken. The first generation of each pneumococcus strain could, as a rule, be regarded as having developed a morphology more or less characteristic for it.

The types of pneumococci regularly met with, excluding involution forms, may be grouped in the following way: the typical form, the small form, the large form, the bacillary form, and the streptococcus form.

The typical form presents lancet or less commonly coccoid-shaped elements enclosed in a characteristic capsule. The capsule takes the form of a deeply staining peripheral ring, elliptical in contour, and separated from the body of the organism by a considerable interval. If we designate this outer ring as the "capsular membrane," the substance included between it and the cocci may be termed "capsular substance." The latter may remain perfectly clear and unstained, or take on a faint color. When these forms are met with in culture, degenerated and empty capsules as well as typical chains are frequently present.

The degenerated capsules stain poorly, or may present tears in their peripheral membrane. The empty forms also stain faintly. Some of them apparently contain diplococci; others have lost one of the two cocci. The capsule at the end of a chain may be empty, one, two, or more cocci being absent. When the pneumococci come into contact there is often a fusion of the capsules, with a disappearance of the "capsular membrane" where they join. The typical pneumococcus chain is composed of capsule and elements similar to those described for the diplococcus. Usually a slight constriction between the diplococci is to be seen. The cultures of pneumococci vary considerably as to the presence of the degenerate and empty capsules and chain forms. At times typical encapsulated diplococci preponderate; at other times many of the other varieties are present in large numbers.

The small form possesses a capsule which is narrow, with a more delicate

contour, and with a capsular membrane approaching the body of the organism more closely. Many strains show this type in the very first generations. The larger pneumococci just described may degenerate into smaller forms after a number of generations, even on the best culture media. When transplanted on dry or unfavorable media they often present this picture.

The larger forms are characterized more by the size and development of their capsules than by any marked increase in the size of the enclosed cocci. The capsules are broader, not so well limited, and more diffusely staining than the usual type. Further, they have a tendency to break up in the process of preparing the specimen, due to their greater viscosity. This type of organism may resemble the diplococcus forms of the Streptococcus mucosus.

In a number of instances I have found strains whose members are elongated so as to form veritable bacilli. Many of these are due to fusion of smaller elements. However, this mode of origin of the bacillary forms is often not apparent. The capsules are generally narrow and diffusely stained. The type belongs to the luxuriantly growing pneumococci. It may appear in the first generations directly isolated from the mouth, or in the later generations of the "large," more mucoid type. Short bacillary forms are very common. The typical lancet-shaped cocci, if slightly elongated, are of this variety.

The streptococcus type is characterized by elements which are round or irregular in shape. Capsules may be absent. When they are present they tend to form delicate membranes situated very close to the cocci and to surround the single members rather than the pairs. I have elsewhere referred to this type of capsule as the "streptococcus type." [1] At times even the first generations show this arrangement. These encapsulated chains are frequently found as degenerations of pneumococci which have been transplanted a great number of times or have been cultivated on unfavorable media.

The following table includes the "completely studied" pneumococci. The characteristic morphology in the first generation and especial points in morphology which manifested themselves in later cultures are indicated.

TABLE XIII.

MORPHOLOGY OF THE PNEUMOCOCCI.*

E 1, typical †
2, " ; or elongated.
3, "
4, "
5, typical on Loeffler; early cultures on serum-agar were of the bacillary type; four months after isolation typical forms were found.
6, typical.
7, small.
8, typical.
9, "
10, "
11, typical; at times elongated, and bacillary.
12, typical.
13, "
14, "
15, typical; or elongated.
16, " " "
17, typical.

* For the sake of reference a number of streptococci are included in this list.

† "Typical," "small," etc., refer to the types of organisms. Whenever nothing to the contrary is stated the encapsulated form is to be understood.

[1] *Proceedings of the New York Pathological Society*, 1904, iv, 131.

TABLE XIII (*Continued*).

18, typical; or elongated.
19, " " "
20, " " "
21, typical.
22, typical; or elongated.
23, typical.
24, "
25, "
26, small; streptococcus form with its typical capsule.
27, typical.
28, "
29, "
(30, Streptococcus mucosus.)**
(31, atypical form.)**
32, typical.
33, "
34, "
35, "
36, typical; large capsules.
37, large, mucoid-type.
38, " " "
39, typical.
40, mostly non-encapsulated; some chains with streptococcus type of capsule.
(41, Streptococcus mucosus.)
(42, atypical organism.)**
43, small; or streptococcus type with the streptococcus type of capsule.
44, typical.
45, small; or streptococcus type with the streptococcus type of capsule.
46, atypical; but with large capsules; involution forms with well developed capsules.
47, typical.
48, "
49, "
50, small.
51, "
52, typical and elongated.
53, typical.
54, many pneumococcus type of chains; and typical forms.
55, typical.
56, "
(57, streptococcus.)**

** Considered in the section on "allied" organisms.

From the above table it will be seen that most of the pneumococci showed the typical encapsulated or slightly elongated forms. A few showed the small and bacillary type, and two the large mucoid forms. The streptococcus variety was found in three cultures. Although the particular morphology above indicated in the table was noted in the first generation of each culture, either on the Loeffler serum or the serum-glucose-agar plates, it must be mentioned that many strains either lost their capsules entirely after a number of transplantations or became converted into other types. It appeared that most of them could assume the smaller or streptococcus type under certain conditions of growth. The bacillary and large forms were at times replaced by the typical forms. None of the varieties, however, changed their morphology to the large mucoid type. Degenerative and involution changes occurred in some cultures of all the strains.

The cultural properties.—All the completely studied organisms were inoculated on the following culture media: broth neutral to phenolphthalein, neutral agar, Loeffler's blood-serum, gelatin, litmus milk, and Hiss' inulin-serum water.

Broth: All the strains with the exception of E 8 caused visible growth in

the bouillon. The luxuriance and the rapidity of growth varied greatly. Some strains (E 5) developed a heavy and diffuse turbidity in twenty-four hours; others produced only the faintest cloud. Very few pneumococci caused the formation of flakes in either turbid or clear bouillon.

Agar: Most of the organisms grew poorly on this medium. E 37 and 38 gave very profuse growths. They presented appearances characteristic of Streptococcus mucosus.

Loeffler's blood-serum: The usual type of growth characteristic for pneumococci was generally obtained. There was considerable variation as to the luxuriance and watery character of the surface growths. E 5, 37, and 38 developed rapidly; the first gave watery, and the other two mucoid, colonies. Some cultures, however, were rather attenuated and their colonies fairly dry.

Gelatin: At 24° C. most of the strains finally gave evidences of growth. At best the colonies were very fine and the development extremely sparse. There was never any liquefaction. The following organisms gave no perceptible growth: E 11, 12, 13, 20, 29, 45, 53, and 54. All of these, with the exception of E 45 and 54, were virulent for white mice. The statement made by some observers, to the effect that the avirulent pneumococci do not develop on gelatin (at the ordinary temperatures) whereas the virulent forms do, could not be confirmed.

Litmus milk: The time required for acid production to manifest itself and for coagulation to take place were noted in each instance. The results are given in the following table.

TABLE XIV.

GROWTH IN LITMUS MILK.

E.	ac.*	coag.†	E.	ac.*	coag.†	E.	ac.*	coag †
1	1	3	19	1	2	39	1	2
2	"	4	20	"	"	40	"	"
3	"	"	21	"	"	42‡	"	3
4	"	"	22	½	1½**	43	"	—
5	"	2	23	2	3	44	"	2
6	2	10?	24	1	4	45	"	"
7	1	4	25	"	"	46	"	3
8	"	—	26	"	2	47	"	2
9	"	2	27	"	"	48	"	4
10	2	3	28	"	"	49	"	1
11	1	2	29	"	"	50	"	2
12	"	"	32	"	"	51	"	3
13	"	3	33	"	1	52	"	"
14	"	2	34	"	2	53	"	"
15	"	1½	35	"	"	54	"	—
16	"	1	36	"	"	55	"	2
17	"	2	37	"	"			
18	"	"	38	"	3			

* ac. = acid in as many days as indicated. † coag. = coagulated.
** About. ‡ Atypical organism; see section on allied organisms.

Two or three days was the usual time required for coagulation. Three strains, E 8, 43, and 54, produced a moderate amount of acid but no coagulation.

Inulin-serum water: Table No. XV shows in how many days coagulation set in.

TABLE XV.

GROWTH IN INULIN-SERUM WATER.

E.	coag.	E.	coag.	E.	coag.	E.	coag.
1	4	15	2	29	2	45	2
2	"	16	1	31*	5	46	"
3	"	17	2	32	2	47	"
4	"	18	4	33	1	48	"
5	"	19	2	34	2	49	5
6	2	20	"	35	"	50	2
7	"	21	"	36	"	51	3
8	"	22	1	37	3	52	2
9	5	23	4	38	"	53	"
10	2	24	1	39	2	54	5
11	1	25	3	40	3	55	2
12	3	26	"	42*	5		
13	2	27	2	43	3		
14	"	28	5	44	2		

* Atypical organism.

Coagulation as a rule took place in from two to four days. Acid production could often be noted in one or two days. Although this medium was in main sufficiently favorable for the growth of pneumococci, a number of lots of serum water were made up which had to be discarded. These last were found to furnish a poor medium for the development of the organism. It is difficult to determine the reasons for the variations in the quality of the medium. An attempt was made to use beef-serum which was free from red blood cells and in which very little, if any, hæmolysis had taken place. The various lots were tested by inoculation with luxuriantly growing pneumococci. If acid production and coagulation were deferred for a number of days the medium was replaced by a fresh lot.

I found that the serum-water medium may be made more favorable for the growth of pneumococci by the addition of peptone. A modified serum-water medium was prepared as follows: Hiss' serum-water mixture is first made up. One hundred c.c. of beef-serum and 200 c.c. of distilled water are put into a flask and steamed for ten minutes. A peptone solution is prepared by dissolving 6 grams of Witte's peptone in 25 to 30 c.c. of water over a small flame. When dissolved it is filtered and cooled. The peptone mixture is then added to the serum water. To this medium as a basis 1 % of inulin and sufficient litmus are added in the usual way. Kahlbaum's litmus has given me the most satisfactory results. Both the peptone solution and the serum water should be cold before mixing. The medium is sterilized for 15 minutes on three or four successive days. After sterilization it has a thin jelly-like consistency. Acid production by pneumococci is usually manifested in 18 to 24 hours.

All of the pneumococci studied fermented inulin.[8] The action of certain

[8] A number of strains failed to ferment inulin in certain generations. Others caused only a suspicion of acid in the inulin-serum water, but gave a more marked reaction medium as modified by me.

organisms resembling the pneumococcus on inulin will be considered in a separate section.

Colonies: I wish to call attention to a number of points regarding the colonies of the pneumococcus. I have already referred to the characteristic "ring" appearance that many strains present. As seen in the serum-glucose-agar plates, the "ring-forms" vary both in size and structure. Thus a central umbilication is sometimes to be discerned on colonies as small as the period employed in ordinary newspaper type. On the other hand, I have occasionally seen the colonies attain a diameter of 1.5 to almost 2 mm. The colonies of this type, when dense, lack the characteristic milky appearance given to them by transmitted light. They are then more mucoid and whiter in appearance. The central umbilication may not become manifest until 48 hours have elapsed, so that a slightly convex watery or mucoid colony may show the typical appearance if it be incubated or allowed to remain at room temperature 24 hours longer.

There are both small and large colonies which do not show the ring form. The small colonies are slightly convex, glistening, and of varying opacity. They may be watery or even somewhat mucoid. Very large colonies are occasionally observed. These may be mistaken for the colonies of the Streptococcus mucosus capsulatus. Their development is just as great and general characteristics the same. The colonies of E 37 and E 38 attained a diameter of 2.5 mm. on the surface of the plates. Both strains were isolated from a case of pneumonia. As far as the macroscopic appearance of these cultures was concerned they were indistinguishable from the mucoid streptococcus. E 33 also gave colonies almost as large as those just described. After a number of transplantations all three strains showed a marked diminution in the luxuriance of their growth on serum media.

PATHOGENICITY OF THE PNEUMOCOCCI.

The question of the virulence of the organisms isolated from the mouths of the "completely studied" cases was determined by inoculating white mice with approximately uniform doses of the pure cultures. The rapid diminution in virulence which the pneumococcus suffers after a number of transplantations, the difficulty in employing exact dosage, and the slight variations in the weight of the mice employed, are facts which affect the interpretation of the results. It was possible, in most instances, to inject pure cultures but one generation removed from the original isolation. Of fifty-one (51) pneumococci derived from the mouth, there were five in which later generations were used. The five strains, however, were virulent even after the second generation. These studies, therefore, may be regarded as a fair index of the pathogenicity of the organisms.

The usual dose was a twenty-four-hour growth on a serum-agar slant. This was suspended in 1 c.c. of normal salt solution and inoculated subcutaneously in the back of a white mouse. Whenever the results were negative or doubtful, as, for example, the possible death of the mouse from some other cause, fresh animals were employed, or larger and repeated doses were sometimes injected into the same animal. In some instances the attempt was made to convert avirulent into virulent strains.

The following table deals with the virulence of fifty-one pneumococci isolated from the mouth:

TABLE XVI.

VIRULENCE OF THE PNEUMOCOCCI.

Strain.	Period of Survival.	Generation	Strain.	Period of Survival.	Generation
E 1	1 day.	2d	E 27	1 day 16 hrs.	2d
2	1 " 21 hrs.	"	28	1 " 12 "	"
3	3 days	3d	29	1 " 15 "	"
4	2 " 2 hrs.	2d	32	— — 22 "	"
5	1 day 12 "	"	33	— — 15 "	"
6	avirulent.	"	34	— — 22 "	"
7	"	"	35	2 days 12 "	"
8	1 day 18 hrs.	"	36	1 day 14 "	"
9	" " " "	"	37	— — 22 "	"
10	avirulent.	"	38	3 days 12 "	3d
11	1 day 16 hrs.	"	39	— — 18 "	"
12	" " 18 "	"	40*	(— — 19? "	8th)
13	" " 21 "	"	43	avirulent.	2d
14	— — 20 "	"	44	— — 23 "	"
15	2 days 12 "	"	45	avirulent.	"
16	" " 16 "	"	46	1 day 18 hrs.	"
17	3 " 12 "	"	47	avirulent.	"
18	avirulent.	"	48	1 day 14 hrs.	"
19	4 days 12 hrs.	"	49	avirulent.	"
20	1 day 24 "	"	50	2 days 15 hrs.	"
21	2 days 19 "	"	51	1 day 16 "	"
22	avirulent.	"	52	— — 22 "	"
23	9 days 16 hrs.	"	53	1 day 2 "	"
24	avirulent.	"	54	avirulent.	"
25	1 day 18 hrs.	3d	55	2 days.	"
26	3 days 12 "	2d			

* Doubtful result; additional mice were negative.

The virulent strains varied considerably in the rapidity with which they brought about the death of the animal. The shortest time was 15 hours. Most of the mice, however, succumbed within two or three days. The pathological changes consisted of fibrinous exudate with varying amounts of hæmorrhage and œdema at the site of inoculation, and now and then increased

peritoneal or pleuritic fluid. The typical encapsulated diplococci were found regularly in films made from the exudate and the blood. In a few instances the diplococcus was also present in spreads made from the peritoneal or pleural fluid. At times, especially in the case of strains E 37 and 38, the exudate was of a mucoid character, but never as gelatinous as in the case of the Streptococcus mucosus capsulatus. When death did not set in rapidly (23) more marked lesions were observed, such as peritonitis, fibrinous perihepatitis, pleuritis, and slight splenic enlargement. Congested or hæmorrhagic areas in the lungs were but rarely seen.

Cultures were taken from the blood and local exudate of every mouse which died of the infection, and the organisms again identified by the capsule stain.

The avirulent pneumococci, although injected in much larger doses, produced no appreciable symptoms in the test animals. In each of these cases at least two mice were employed. In a number of the eleven negative instances intraperitoneal or subcutaneous injections into mice were employed, and the animals killed in eighteen to twenty-four hours. Capsule stains were then made. Thus E 40 was avirulent for most of the animals and did not present the typical morphology on the plates, but it showed non-encapsulated and degenerate forms of the pneumococcus as represented by chains with or without the "streptococcus type" of capsule. An inoculated mouse killed twenty-four hours after injection showed typical but small encapsulated pneumococci.

Of the total of fifty-one cultures of mouth pneumococci, eleven were avirulent and forty virulent, or 21.5 % and 78.5 %, respectively. In the cases of the "normal" mouth cultures, eight were avirulent out of thirty-eight, or 21 %. Of thirteen cultures from the pneumonia cases, three were avirulent, or 23 %. Apparently, therefore, about the same proportion of non-virulent pneumococci are to be found in the mouths of patients suffering from pneumonia as in the mouths of normal individuals.

Pneumococci occurring in the mouths of normal individuals are, as far as we have been able to determine, identical with those occurring in the mouths of patients with pneumonia and obtained from other sources. A few minor differences were noted. Pneumococci isolated from the mouth grow more rapidly and more luxuriantly than those isolated from pus of empyæma. The elongated almost bacillary types with well-

formed capsules occurred more frequently in the recent cultures from "normal" cases. This latter peculiarity appears to be an expression at times of high development under favorable cultural conditions. In regard to virulence and the lesions produced in white mice no differences were to be noted. Some strains from the "normal" mouth retained their pathogenicity quite as long as the strains from other sources. Thus culture E 5, from a "normal" case, was still virulent almost four months after isolation.[9]

SUMMARY ON THE OCCURRENCE OF PNEUMOCOCCUS.

1. Certain characteristic morphological types of pneumococcus were met with regularly in the course of this study.

2. The pneumococci occurring in the mouths of normal individuals possess the usual morphological and cultural characters observed in the organisms isolated from other sources.

3. All the pneumococci studied possessed the power of splitting inulin with the formation of acid.

4. The percentage of virulent organisms found in the mouths of normal cases was 79; in pneumonia cases 77.

ORGANISMS IN THE MOUTHS OF NORMAL INDIVIDUALS WHICH CLOSELY RESEMBLE THE PNEUMOCOCCUS.

In this section will be considered certain organisms which belong to the pneumococci but present variations from the usual type, organisms related to the pneumococci but possibly intermediate in development between the streptococci and pneumococci, and the Streptococcus mucosus capsulatus.

Certain atypical pneumococci:—Culture E 40 presented none of the typical capsulated forms of the pneumococcus at the time of isolation, but non-

[9] In ten cases the saliva of the patient was taken for direct animal inoculation at the same time that plates were made. The intention was to compare the value of the plate method and animal inoculation in recovering the pneumococcus. By the latter means four positive instances were obtained. In each of these the pneumococcus was isolated in pure culture, ready for the test inoculation, at the end of forty-eight hours. Only three positive cases were secured by direct animal injection. It was, moreover, necessary to plate out the organism from the blood of the infected animals in order to obtain pure cultures. It would seem, therefore, that more reliable and rapid results are to be obtained not by inoculation but by the plate method.

capsulated diplococci and chains with the streptococcus type of capsule. Several mice inoculated with early generations of the organism were not affected One mouse died nineteen hours after inoculation with the eighth generation, but as no organisms were found in the blood this result was regarded as doubtful. Typical, although poorly developed, capsular forms were, however, discovered in a mouse killed for the purpose of diagnosis.

This organism was found variable in its action on inulin. A number of tests were made with different generations and with a strain isolated from a mouse. After three days there was slight coagulation with moderate amount of acid production. Complete coagulation did not take place. At times no appreciable acid was formed. One tube showed the reaction at the end of six days. The distinguishing features of this organism were: atypical morphology, non-virulence, and variable fermentative power for inulin.

Of the pneumococci with large mucoid colonies three cultures were isolated from the mouth. A similar strain was cultivated from the exudate in a case of early empyæma. These pneumococci are to be distinguished from the Streptococcus mucosus by their morphology. Their elements are regularly lancet-shaped in the early generations. Their chains are of the pneumococcus type, i. e., composed of pairs of lancet-shaped cocci surrounded by a capsule which shows a tendency to slight constriction between the diplococci. The Streptococcus mucosus under the same conditions will be found to possess either round or biscuit-shaped elements, and the capsule about its chains never shows the indentation so common in the case of pneumococci. The luxuriance of growth of these mucoid pneumococci may diminish rapidly after a number of transplantations. The Streptococcus mucosus, on the other hand, does not seem to undergo much change.

Organisms related to the pneumococcus:—Culture E 31 was isolated from the mouth of a so-called normal patient. Its morphology was peculiar and quite different from that of the ordinary pneumococcus. Specimens stained with the capsule method, and taken from cultures on serum-agar, or Loeffler's medium, presented the following picture. Most of the elements were grouped in pairs, and were coccoid or slightly elongated. Often single cocci or short bacillary forms with rounded ends were to be seen. The capsules when they enclosed two elements often showed deep constrictions between the cocci, so that the latter appeared almost separated. Another feature was the tendency for the organisms to clump. When aggregated in this way, the contiguous capsules fused at the lines of contact, so that a single enveloping membrane for a whole clump could be seen. Even considerable manipulation in the process of spreading failed to separate them completely.[10] In neutral or serum-bouillon long chains were formed.

The following was noted in cultures: Milk was coagulated in five days. With inulin it behaved variably. The early cultures caused fermentation, the later ones fermented slowly or not at all. Mouse No. 1 received four inoculations without effect. Mouse No. 2 died in forty-three hours; there was a fibrinous hæmorrhagic exudate locally. No typical pneumococcus forms were found either in the exudate or in the blood. Mouse No. 3 did not succumb.

[10] This appearance became somewhat altered after long cultivation.

Mouse No. 4 was killed for diagnosis, but no organisms were detected in the spreads. This strain was therefore of doubtful virulence. The features of the organism were: peculiar, atypical morphology; irregular action on inulin, and non-virulence for mice.

Still another type (E 42) resembling the pneumococcus was isolated from a "normal" patient. On Loeffler-serum and serum-agar media it grew as diplococci which showed very delicate eccentrically placed capsules. Usually only one half of the capsule was discernible. Careful adjustment of the focus, and the use of a high ocular, often enabled one to see the other side of the capsule as a faint linear envelope situated very close to the bodies of the diplococci. A similar appearance was now and then to be seen in the case of short chains.

Cultural characters: Milk was coagulated in four days, neutral bouillon was diffusely clouded; on gelatin there was very poor growth. Variable results were obtained on the inulin medium. Two of the early cultures coagulated the inulin-serum water in four and five days respectively. At other times only a very little acid, or none at all, was produced. Animal inoculations were negative. The typical pneumococcus form was not formed even after a number of animal inoculations. This organism may therefore be regarded as having features in common both with the streptococcus and the pneumococcus.

Streptococcus mucosus capsulatus:—A number of organisms belonging to this group were isolated from normal mouths. They form a distinct class by themselves and should be differentiated from pneumococci, although the latter, both in their cultural properties and morphology may often closely resemble them.

In the blood and exudates of infected animals their characteristic morphology is developed. In these the Streptococcus mucosus takes the form of diplococci or chains surrounded by a particular type of capsule. The elements are usually somewhat biscuit-shaped. However, they may be coccoid, slightly elongated, or even lancet-shaped. The lancet form is rare, and when it is found is usually associated with many of the other forms. The capsules of the diplococci are almost circular in outline, very large and broad, with a tendency to stain diffusely. Those surrounding the chains give no evidence of having been formed by the fusion of smaller elements. The outer membrane shows none of the indentations which are commonly seen on the pneumococcus chains. In the blood and exudates of infected animals the diplococcus form usually preponderates. Short chains of four are very common and chains of six or eight elements may be found.

On culture media, especially on the serum media and Loeffler's coagulated blood-serum, the same morphology is to be observed. Although the capsules of the streptococcus are fairly resistant in the early generations taken from animal exudates, in general they are more easily broken up, more mucoid, and less well defined in their outlines than those of the typical pneumococcus. Involution forms, longer chains, and smaller and more membranous envelopes usually occur in the later generations.

The colonies of these organisms on the plates often attain considerable dimensions. As a rule, they measure about 1.5 to 2 mm., are mucoid and glassy by reflected light, milky and more opaque by transmitted light. Colonies measuring 3 mm. or slightly more in diameter are occasionally seen. If the plates are allowed to remain at room temperature for a second 24 hours after incubation,

an appearance similar to that presented by the "ring type" of pneumococcus colonies is often developed. The centers of the colonies become clear and the peripheries stand out as milky annular bands.

On the surface of the serum-agar or Loeffler's medium there is a glistening, mucoid, apparently transparent growth. Discrete colonies may be likened to drops of water. By transmitted light the opacity of the colonies can be seen.

Milk and the inulin-serum water are coagulated rapidly. The growth on gelatin is poor, and there is no liquefaction.

The organisms studied were virulent for white mice, death usually resulting in 20 to 48 hours. After subcutaneous inoculation a mucoid, almost gelatinous exudate, often with considerable œdema and hæmorrhage, was produced locally. The typical encapsulated organisms were present in the exudate and in the blood.

Although the very profuse and characteristic growth of this organism on serum-agar or serum-glucose-agar usually enables one to differentiate it from the pneumococcus, this is not always the case. Reference has already been made to three strains of pneumococci as they grew on the above mentioned media which were indistinguishable from the Streptococcus mucosus. For the diagnosis one must rely upon morphological differences. The forms in the blood and exudate of animals inoculated with the two types of organisms show the same differences in morphology as do their cultures on favorable media. After a number of transplantations, the pneumococci may suddenly lose their luxuriance of growth, while the mucoid streptococci vary but little in this respect. In older cultures the distinction of the organisms by means of their morphology presents no difficulties.

RÉSUMÉ OF THE FACTS RELATING TO IDENTIFICATION AND CHIEF DIAGNOSTIC FEATURES OF THE PNEUMOCOCCUS.

The identification and isolation of the pneumococci were accomplished by the plate method and on Loeffler's serum. The organisms which could be identified as pneumococci by means of their morphology were (*a*) typical encapsulated forms, (*b*) small encapsulated forms, (*c*) large encapsulated forms, (*d*) bacillary encapsulated forms; other organisms encountered were non-encapsulated diplococci or chains [11]; encapsulated streptococci, i. e., streptococci with the streptococcus type of capsule; diplococci or chains with atypical capsules, or with other peculiar morphological characters; streptococci with mucoid capsules (Streptococcus mucosus capsulatus).

Pneumococci classified as "typical," "small," large," or "bacillary," when virulent, regularly presented the typical morphology in the blood and exudates of white mice. Positive animal tests were regarded as furnishing conclusive evidence as to the nature of the organism. From a study of a large number of strains it was found that organisms of the above morphology always belonged to the pneumococci. Even when not virulent it was possible to demonstrate the typical encapsulated forms in animals.

[11] Any of these *typical varieties* (*a*), (*b*), (*c*), and (*d*) may grow in chains, and possess the characteristic capsule. The word "chains" as used here does not refer to these types.

The value of the morphological identification on Loeffler's serum was investigated by plating out about twenty-five cultures and controlling the results in that way. Pure cultures of pneumococci were obtained in all of the tubes in which encapsulated pneumococci had been recognized in the films.

In a separate study it was found that pneumococci frequently lost their capsules after a number of transplantations or when cultivated on unfavorable culture media. Non-encapsulated chains resembling streptococci were also met with under these conditions. The question arose, therefore, as to whether the organism could possibly lose its typical form in the first generation on Loeffler's blood-serum or on serum-glucose-agar plates, and take on the form of non-encapsulated diplococci and chains. A large number of these forms were therefore isolated and studied both culturally and by animal inoculation. None of these could be converted into the encapsulated types or identified with the pneumococcus by cultural or other tests. It would seem, therefore, that pneumococci isolated from the mouth and growing on the media employed, regularly take on the encapsulated form in the first generation.

Encapsulated streptococci with spherical or biscuit-shaped elements and surrounded by a membranous, linear envelope[12] were more frequently met with especially on the Loeffler serum. These occur frequently as degeneration forms of pneumococci, but may belong to the streptococci. A number of such streptococci were isolated and studied. E 26 and 45 showed this character, but their identification was easy on account of the presence of the "small" encapsulated pneumococcus forms. E 40, however, showed none of the varieties of diplococci which are at once recognizable by their morphology. There were many encapsulated chains of the "streptococcus type," but the typical diplococcus form was obtained in a white mouse. Of the many other encapsulated streptococci isolated and studied none could be proven to be pneumococci, either culturally or by animal inoculation. There was, therefore, only one instance (E 40) in which the pneumococcus did not show some one or other of the typical pneumococcus forms in the first cultures. Possibly poor quality of the medium might explain this case.[13]

There seems little doubt that there are many doubtful diplococci of atypical morphology possessing characters in the stained specimens which are somewhat different from those of the ordinary pneumococci, and yet in cultures they may exhibit characters resembling both the pneumococci and the streptococci. E 31 and 42, which have already been described at length, differed from the pneumococci in their morphology and resembled them in their ability to ferment inulin. Animal inoculations in these cases failed to give any positive evidence of their pneumococcal nature. One strain resembling E 31 and another resembling E 42 had previously been isolated but not completely studied. At the present time I am unable to classify these two strains. Perhaps they are intermediate organisms between streptococci and pneumococci. Possibly E 42 approaches nearer the streptococci and E 31 the pneumococci.

The Streptococcus mucosus belongs to a group which is related to, but distinct from, the pneumococcus. Its recognition by means of morphology is usually easy.

---

[12] I. e., the "streptococcus type" of capsule.

[13] E 57 was a streptococcus which showed a narrow capsule.

As one becomes acquainted with the various types of pneumococci when stained with the capsule method, their differentiation from other encapsulated organisms, such as the Micrococcus tetragenus, the diplococcoid forms of the Friedländer bacillus, and the encapsulated diplococcus often found in beef-serum, presents little difficulty. The capsules of Micrococcus tetragenus are usually small,[14] and the cocci themselves large and round or flattened. The Friedländer bacillus is identified by the employment of the combined "Gram and capsule" stain. The organism found in beef-serum is a somewhat elongated stout coccus which is much larger than the pneumococcus, and has a narrow membranous capsule.

Perhaps the most valuable cultural features in the diagnosis of the pneumococcus are the fermentation of inulin, the growth on serum-glucose-agar, and the appearances of the colonies.

In a study of the streptococci it was found that a certain number of these are able to split inulin with the formation of acid. The present work confirms the view of Hiss that all pneumococci, as far as known, ferment this carbohydrate. Some pneumococci, however, fail to produce acid or coagulate the inulin-serum water in certain generations. This variation has been frequently noted, and to a certain extent impairs the value of the test.[15] Continued failure to ferment inulin would seem to speak against an organism being the pneumococcus, while the possession of the fermenting power cannot be regarded as proving it to be.

Finally, as regards the serum-glucose-agar medium, it may be added that practically all streptococci caused precipitation or whitening of it. The pneumococci, on the other hand, with very few exceptions, showed no tendency to produce this change.[16]

AGGLUTINATION OF THE PNEUMOCOCCI AND ALLIED ORGANISMS.

In this section I will present my observations on the agglutination of many of the pneumococci described in the previous sections. Unfortunately, time did not permit an exhaustive study of this phase of the subject. Although a sufficiently large number of strains were studied, only a very small number of sera could be obtained. The study embraced the following topics:

Agglutination of pneumococci by immune serum.

Agglutination of allied organisms and streptococci by the serum of animals immunized against the pneumococcus.

[14] I have recently isolated a strain of the Micrococcus tetragenus, whose capsules are of the large mucoid type. This organism is, however, easily recognized.

[15] The variations in the quality of the medium and the growth of the culture must be taken into consideration. A single negative experiment is of little value; it should be confirmed by repeated tests.

[16] Libman, *op. cit.*, has made similar observations.

Agglutination of pneumococci by means of the serum of patients with pneumonia.

Agglutination of pneumococci from so-called normal mouths by the serum of the persons from whom they were isolated.

Agglutination of the pneumococci with immune serum.—For the purpose of immunization pneumococci from two sources were selected: strains isolated from pus from an empyæma, and from the "normal" mouth. Rabbits were inoculated intravenously or intravenously and subcutaneously together. The cultures injected consisted of serum-agar growths incubated for twenty-four hours, and suspended in normal saline.

Six rabbits were immunized with as many different strains. Two of these succumbed after several weeks. Rabbit No. 1 was inoculated during a period of four months and showed marked emaciation. Its serum never developed any marked agglutinating power. The same was true of all the animals, with the exception of rabbit No. 3. The serum of this animal was found to have attained a higher value than any of the rest, and was selected for most of the reactions. Culture E 5 was employed for the immunization of this animal. After a period of about two months and a half this rabbit also succumbed. Sufficient serum had been obtained to carry on a number of experiments.

In the preparation of cultures for agglutination it was decided to employ bacterial suspensions in normal (0.85 %) saline rather than broth or serum-broth cultures. After considerable experimentation the following procedure, similar to one employed by Wadsworth,[17] was adopted.

Small flasks (or a number of large tubes) of serum-bouillon [18] (neutral bouillon 3 parts, ascitic serum 1 part) were inoculated and incubated for twenty-four hours. The bacterial cells were then centrifuged, washed twice by means of normal saline, and the sediment thoroughly shaken up in some of this fluid. Inasmuch as there was considerable variation in the luxuriance of growth of the various strains, it was not possible to adopt any hard and fast rules as to the quantity of the salt solution to be used for the emulsion. The proper density of the suspensions having been determined by a series of tests, fresh tubes of suspensions were kept on hand as controls or standards.

Serum-bouillon was selected in preference to the plain broth because it was found to be much more favorable for the growth of the organisms. Although the early generations, and recent isolations from animals, regularly gave fairly luxuriant cultures in plain broth, the later cultures often developed poorly. Most of the strains had been kept alive on artificial media for a considerable time before agglutination tests were made. In the serum-broth no albuminous precipitation could be detected at the end of twenty-four hours. Microscopic examination of the saline suspensions showed practically nothing but the bacterial cells. It was found, however, that the serum-bouillon was not always suitable for this work. Certain of the pneumococci did not produce a diffuse

---

[17] *Jour. of Medical Research*, 1903–04, x, 228.

[18] The broth was made from meat infusion which had been neutralized before boiling, and contained 2 % peptone.

turbidity in this medium, but grew with the formation of dense flocculi.[19] The flakes being very difficult to break up, the process of washing, sedimenting, and suspending failed to separate the clumps completely. Suspensions made from these cultures had therefore to be discarded.

Pneumococci which grew in flocculi were cultivated on a number of serum-agar slants, from which the surface growth was suspended in saline and treated in the same way as the bouillon cultures. A satisfactory distribution of the organisms was obtained in this way. A number of tests sufficed to show that the agglutinability of the agar- and serum-bouillon cultures was practically the same.

The macroscopic reactions were made in the usual way. One half c.c. of a sufficiently dense suspension was added to a similar volume of the dilute serum. Dilutions were made up to 750 or 1000, the tubes incubated at 37° C., and observed for twenty-four hours. Generally the macroscopic reactions are not absolutely trustworthy. The clumping very often does not manifest itself in the higher dilutions until eight to twelve hours have elapsed. At about this time a certain amount of sedimentation by gravity may occur in the controls. At the end of twenty-four hours this has become even more marked, so that it is often impossible to decide as to the value to be put on the test. Moreover, the clumps may be so fine as to escape observation. This may interfere with the decision both as to time and to the dilution in which the reaction sets in. The microscopic reactions are not open to these objections.

The microscopic reactions were made in every instance, very often side by side with macroscopic tests. At least two sets of reactions were made with each organism. In one the dilutions were freshly made, and in the other stock dilutions of the serum were employed. The results were practically the same in both instances. A small drop of the culture was mixed with a small drop of the dilute serum on a cover-slip for examination by the hanging-drop method. These slides were incubated just as were the tubes. Microscopic examinations of the contents of the tubes were also made. The results of these often differed considerably from the macroscopic readings.

On account of the limited amounts of serum and the large number of pneumococci to be tested, I was unable to carry out the macroscopic tests on all the strains. A sufficient number were, however, made to assure me that the microscopic tests were in main just as reliable as the macroscopic ones.

The reactions with the serum of Rabbit 3 alone will be given. A month after the first intravenous inoculation, the serum of this animal agglutinated neither streptococci nor pneumococci. Gradually increasing agglutinative power was developed. Just before the death of the animal, the serum had attained its maximum power. The rabbit was bled while moribund, the blood collected, and the serum stored in the refrigerator. At that time it was tested with the homologous pneumococcus E 5.

[19] The same is true of the plain neutral broth.

TABLE XVII.

MACROSCOPIC AGGLUTINATION WITH IMMUNE SERUM.

	P 1.			P 15.			E 3.			E 4			E 5		
	4h.	12h.	24h.	4h.	12h.	24h.	4h.	12h.	24h.	4h.	12h.	24h.	4h.	12h.	24h.
1:25	− + + +	+ + +	+ + +	+ + +	− + + +	+ + +	-- + + +	+ + +	+ + +	+	+	+ +	+ +	+ + +	− + + +
1:50	− + + +	+ + +	+ + +	+	− + + +	+ + +	+ + +	+ + +	+ + +	+	+	+ +	+	+	+ + +
1:75	− + + +	+ + +	+ + +	−	+	+ +	+ + +	+ + +	+ + +	−	+	+ +	−	+	+ + +
1:100	− + +	+ + +	+ + +	−	−	+	+ +	+ + +	+ + +	−	+	+ +	−	+	+ + +
1:150	−	+ + +	+ + +	−	−	−	+ +	+ + +	+ + +	−	+	+	−	−	+ + +
1:200	−	+ + +	+ + +	−	−	−	+ +	+ + +	+ + +	−	−	+	−	−	+
1:300	−	+ +	+ +	−	−	−	−	+ + +	+ + +	−	−	−	−	−	+
1:500	−	−		−	−	−	−	−	−	−	−	−	−	−	
1:750	−	−		−	−	−	−	−	−	−	−	−	−	−	

P refers to a special series of pneumococci not derived from the mouth. Most of them were isolated from cases of empyæma.

+ + + = complete clumping.

− + + + = almost complete, good clumping; clearing was not complete.

+ + = small clumps.

+ = very fine clumps.

h = hours.

Complete agglutination was obtained in a dilution of 1:150. The study of the greater number of strains was deferred for a month, at which time the value for E 5 had dropped to 1:100.

Additional macroscopic reactions were made with pneumococci E 6 to E 12 inclusive. The results were practically the same as those obtained in the microscopic tests. The following reactions were obtained in from twelve to twenty-four hours.

	Complete.	Incomplete.		Complete.	Incomplete.
E 6	1:75	1:200	E 10	1:50	1:100
7	1:100	—	11	1:50	—
8	1:50	1:150	12	1:50	1:75
9	1:75	1:100			

By the microscopic tests agglutination can often be observed in twenty or thirty minutes. The temperature of 37° C. seems to hasten the process. Partial or even complete reactions may take place in much higher dilutions at 37° C. than at room temperature.

The appearance of true clumping under the microscope is very characteristic. When the agglutination is complete, the diplococci or chains are approximated in irregular areas which are bound together by strands or isthmuses of clumped bacteria. The clumps are not discrete as in the case of the typhoid bacillus. They have a tendency to join by means of narrow offshoots or processes. As a rule, such large masses include practically all the bacteria. More rarely we find a number of free non-agglutinated organisms and small discrete clumps. When agglutination is incomplete, larger or smaller masses of pneumococci are found scattered throughout the field. These features were also found to occur with the streptococci.

In table No. XVIII the results of the agglutination reactions of pneumococci from the following sources are given: (*a*) normal mouths; (*b*) the mouths of pneumonia patients; (*c*) empyæma pus. The readings show the reactions at the end of twelve to sixteen hours. Observations were regularly continued until twenty-four hours had elapsed. Usually there was very little difference, if any, between the sixteen-hour and twenty-four-hour reactions.

The sera of three normal rabbits were tested against fifteen pneumococci and all the streptococci and atypical organisms. The dilutions were made from 1:5 up to 1:750. In no instance was there evidence of any agglutinative reaction in these dilutions.

TABLE XVIII.

MICROSCOPIC AGGLUTINATION OF PNEUMOCOCCI WITH IMMUNE SERUM.

Culture.	1:20	1:40.	1:60.	1:100.	1:150.	1:200.	1:300.	1:500.	1:750.
P 1	+ + +	+ + +	+ + +	+ + +	− + + +	− + + +	− + + +	+	−
P 15	+ + +	+ + +	+ + +	+ +	+ +	−	−	−	−
Ps30*	+ + +	+ + +	+ +	+ +	+ +	−	−	−	−
P 60	+ + +	+ + +	+ + +	− + + +	+	−	−	−	−
P 61	+ + +	+ + +	+ + +	+ + +	+ +	+	−	−	−
P 72	+ + +	+ + +	− + + +	+ +	+ +	+	−	−	−
E 1	+ + +	+ + +	+ +	+ +	+ +	−	−	−	−
3	+ + +	− + + +	− + + +	− + + +	− + + +	− + + +	+ +	−	−
4	− + + +	− + + +	− + + +	+	−	−	−	−	−
5	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+	+	−
6	+ + +	+ +	+	+	−	−	−	−	−
7	+ + +	+ + +	+ + +	+ + +	+ + +	−	−	−	−
8	+ + +	+ + +	+ +	+ +	+ +	+	−	−	−
10	+ + +	+ + +	+ +	+	−	−	−	−	−
11	+ + +	+ + +	−	−	−	−	−	−	−
12	+ + +	+ + +	+ + +	+	−	−	−	−	−
15	+ + +	+ + +	+ + +	+ +	−	−	−	−	−
16	+ + +	+ + +	+ +	−	−	−	−	−	−
17	+ + +	+ + +	+ +	−	−	−	−	−	−
18	+ + +	− + + +	+ +	+	−	−	−	−	−
19	+ + +	+ + +	+ +	−	−	−	−	−	−
20	+ + +	− + + +	−	−	−	−	−	−	−
21	+ + +	+ + +	+ + +	+ +	−	−	−	−	−
22	+ + +	+ + +	+ + +	+ + +	+ +	+	−	−	−
23	+ + +	+ + +	+ +	+	−	−	−	−	−
24	+ + +	+ + +	+ + +	− + + +	+ +	+	−	−	−
26	+ + +	− + + +	+ +	+	−	−	−	−	−
27	+ + +	+ + +	+	−	−	−	−	−	−
28	+ + +	+ + +	− + + +	+	−	−	−	−	−
29	+ + +	− + + +	+ +	−	−	−	−	−	−
32	+ + +	− + + +	+	−	−	−	−	−	−
33	+ + +	− + + +	+	−	−	−	−	−	−
34	+ + +	+ + +	+ +	+	−	−	−	−	−
35	− + + +	− + + +	+ +	+	+	−	−	−	−
36	+ + +	+ + +	+ +	−	−	−	−	−	−
38	+ + +	+ + +	+ + +	+ +	+ +	−	−	−	−
39	+ + +	+ + +	+ + +	+ + +	+	−	−	−	−
40	+ + +	+ + +	+ + +	+ + +	− + + +	+ +	−	−	−
44	+ + +	+ + +	− + + +	+	−	−	−	−	−
45	+ + +	+ + +	+ + +	+ +	+	+	+	−	−
46	+ + +	+ + +	− + + +	+	−	−	−	−	−
47	+ + +	+ + +	+ + +	− + + +	+ +	+	−	−	−
48	+ + +	+ + +	+ + +	− + + +	+ +	+	−	−	−
49	+ + +	+ + +	+ + +	− + + +	+ +	+	−	−	−
51	+ + +	+ + +	+ + +	+	−	−	−	−	−
52	+ + +	+ + +	− + + +	−	−	−	−	−	−
53	+ + +	+ + +	+ + +	+ +	+ +	+	−	−	−
54	+ + +	+ + +	− + + +	+	−	−	−	−	−
55	+ + +	+ + +	+ + +	+ +	+ +	+	−	−	−

* An organism of the large mucoid type.

+ + + = complete; all organisms clumped.

− + + + = " with a few scattered bacteria. + + = discrete large clumps.

+ = small clumps; many pneumococci not agglutinated. − = negative.

If the organisms be grouped according to their source, the results may be tabulated in the following manner:

TABLE XIX.

AGGLUTINATION OF PNEUMOCOCCI FROM THE NORMAL MOUTH.

	Complete.	Incomplete.		Complete.	Incomplete.
E 3	1:200	1:300	E 27	1:40	1:60
4	1:60	1:100	28	1:60	1:100
5	1:100	1:500	29	1:40	1:60
6	1:60	1:100	39	1:100	1:150
11	1:40	1:60	40	1:150	1:200
12	1:60	1:100	44	1:60	1:100
15	1:60	1:100	45	1:60	1:300
16	1:40	1:60	46	1:60	1:100
17	1:40	1:60	47	1:100	1:200
18	1:40	1:100	48	1:100	1:200
19	1:40	1:60	49	1:100	1:200
20	1:40	—	51	1:60	1:100
21	1:60	1:100	52	1:60	—
22	1:100	1:200	53	1:60	1:200
24	1:100	1:200	54	1:60	1:100
26	1:40	1:100	55	1:60	1:200
(From Pneumonia Cases)			(From Empyæma Pus) *		
E 1	1:40	1:150	P 1	1:300	1:500
7	1:150	—	15	1:60	1:150
8	1:40	1:200	30	1:40	1:150
10	1:40	1:100	60*	1:100	1:150
23	1:40	1:100	61	1:100	1:200
32	1:40	1:60	72	1:60	1:200
33	1:40	1:60			
34	1:40	1:100			
35	1:40	1:150			
36	1:40	1:60			
38	1:60	1:150			

* All from empyæma pus, except P 60, which was isolated from the blood of a mouse which had spontaneously acquired a pneumococcus infection.

A consideration of Tables XVIII and XIX appears to teach that although there are considerable variations in the agglutinability of the various strains, these are not sufficiently marked to enable one to separate them into classes. The homologous organism E 5 was completely agglutinated in a dilution of 1:100 and incompletely up to 1:500. Other pneumococci, however, gave just as good or better reactions. Thus E 3 was completely clumped in a dilution of 1:200. Pneumococcus P 1, isolated

from empyæma pus, gave the best reactions. Organisms derived from this source and from the mouths of pneumonia patients were agglutinated in just as high dilutions as those from the mouths of "normal" individuals.

The agglutination of allied organisms and streptococci with the serum of animals immunized against the pneumococcus.—The same immune serum was employed for agglutination tests on the following organisms: atypical intermediate organisms, certain streptococci, and a number of strains of the Streptococcus mucosus capsulatus. The microscopic tests appeared to give very definite results. The picture of the clumping is very similar to the one already described.

The tests on streptococci were made in order to determine whether the immune pneumococcus serum possessed agglutinins for streptococci as well as for pneumococci. The results tabulated below will show that doubtful organisms, the streptococcus and the Streptococcus mucosus capsulatus, were all agglutinated by the serum:

TABLE XX.

AGGLUTINATION OF VARIOUS ORGANISMS.

Streptococci.

Culture.	1:20.	1:40.	1:60.	1:100	1:150.	1:200.	1:300.	1:500.	1:750.
S 5	+++	+++	+++	++	+	+	−	−	−
7	+++	+++	+++	−+++	++	++	++	++	+
9	+++	+++	+++	++	+	+	−	−	−
11	+++	+++	+++	+	+	−	−	−	−
23	++	++	+	−	−	−	−	−	−
31	+++	+	−	−	−	−	−	−	−
80	+++	+++	+++	+++	+	−	−	−	−
E 57	+++	+++	−+++	+	+	−	−	−	−

Atypical "Intermediate" Organisms and Streptococcus Mucosus Group.

Culture.	1:20.	1:40.	1:60.	1:100.	1:150.	1:200.	1:300	1:500.	1:750.
E 31	+++	+++	+++	+	−	−	−	−	−
42	+++	+++	+++	++	+	−	−	−	−
S. M. I	+++	+++	+	+	+	−	−	−	−
II	+++	+++	+++	++	+	−	−	−	−
IV	+++	+++	+++	+	+	+	−	−	−

TABLE XXI.

SUMMARY OF AGGLUTINATION TESTS OF TABLE XX.

Streptococci.			*Atypical Organisms.*		
	Complete.	Incomplete.		Complete.	Incomplete.
S 5	1:60	1:200	E 31	1:60	1:100
7	1:100	1:750	42	1:60	1:150
9	1:60	1:200			
23	—	1:60		*Streptococcus Mucosus.*	
31	1:20	1:40	S. M. I	1:40	1:150
77	1:60	1:150	II	1:60	1:150
80	1:100	1:150	IV	1:60	1:150
E 57	1:60	1:150			

S 5 and S 9 were isolated from empyæma pus; S 7 and S 23 from the blood of cases of "malignant" endocarditis; S 31 from an appendix; S 77 and 80 from the intestinal contents of normal individuals, and E 57 from the "normal" mouth. For E 31 and 42 see Table II. S. M. I was isolated from the cerebro-spinal fluid of a case of cerebro-spinal meningitis, S. M. II from a spontaneously infected mouse, and S. M. IV from a "normal mouth."

It appears, therefore, that an immune pneumococcus serum can agglutinate streptococci, certain atypical organisms, and the Streptococcus mucosus as well as pneumococci. I was unable to study the specificity of the agglutinins by the absorption methods. The tests made showed that the agglutination reaction was of no aid in the classification of the pneumococci; whether or not an immune serum of higher value would have shown greater differences in its reaction towards pneumococci and streptococci I cannot say.

A few experiments were made in order to determine the difference of agglutinability of pneumococci which had been recently recovered from an animal and the old stock strains. At times there was no appreciable difference. At others, the recently isolated organism was clumped in somewhat higher dilutions. Thus E 5 was completely agglutinated in a dilution of 1:150 after passage through one animal, whereas the stock culture gave a reaction of 1:100.

The agglutination of pneumococci by the serum of pneumonia patients.—The sera of three cases were employed for the tests. Two of them were fairly fresh and were obtained from Cases 31

and 45 (see Table II). The third serum, kindly given me by Dr. Libman, had been taken from a very severe case of lobar pneumonia in April, 1904, and was therefore about eleven months old. It had been kept in the refrigerator and was sterile.

The sera from Cases 31 and 45 gave no agglutination reaction either with the homologous organism (from the mouth of the same case) or with a number of other pneumococci.

The old serum, however, still showed considerable agglutinative power. A number of pneumococci were tested with it; some were clumped and others gave no reaction at all, although tested a number of times. The results of some of the reactions are given below:

TABLE XXII.

AGGLUTINATION WITH PNEUMONIC SERUM.

	1:20.	1:40.	1:60.	1:100.	1:150.	1:200.	1:300.	1:500.	1:750.
P 1	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+	+
E 3	+	+ +	+ + +	+	−	−	−	−	−
E 5	+ +	+ +	+ +	+	−	−	−	−	−
P 15	−	−	−	−	−	−	−	−	−
21	−	−	−	−	−	−	−	−	−

It is interesting to note that P 1 gave the best reactions not only with the immune serum but also with the old pneumonia serum. This particular organism had been kept alive for many months, but still retained its encapsulated diplococcus form. P 15, although regularly presenting the typical form, was not at all affected by the serum in a number of tests.

Serum from a case of uræmia and from a normal individual served as controls in these experiments. Neither gave any reaction in a dilution of 1:5.

The agglutination of pneumococci by the serum of normal individuals.—Sera from a number of the so-called normal individuals were obtained by aspiration of large veins in the region of the elbow. Each of these was tested against the organism derived from the mouths of the same patient. Dilutions from 1:5 up to 1:500 were made and both macroscopic and

microscopic tests set up. None of the sera showed any agglutinating power. The tests are given below:

Case 24 *vs.*	E 16 and 19;	result	negative.
" 36 "	E 15, 20, and 22;	"	"
" 39 "	E 18 and 10 *;	"	"
" 32 "	E 11 " 12 *;	"	"
" 41 "	E 17 " 12 *;	"	"

The agglutinability of the particular suspension was controlled by making reactions with immune serum.

* These organisms did not belong to the respective cases, but served as controls.

RÉSUMÉ OF THE WORK ON AGGLUTINATION.

The successful immunization of rabbits against pneumococci is attended with considerable difficulty owing to the marked susceptibility of these animals to a general infection.

The animals elaborate agglutinins slowly and poorly.

All the pneumococci, irrespective of their source, were agglutinated by means of immune serum.

One sample of pneumococcus immune serum was found to agglutinate various pyogenic streptococci, the atypical organisms, and several strains of the Streptococcus mucosus capsulatus.

The serum of a pneumonia patient varied as to its power to agglutinate different pneumococci. Some strains were agglutinated, others not.

The sera of normal human individuals and of normal rabbits possess no agglutinating power for pneumococci, the atypical organisms, certain streptococci, and the Streptococcus mucosus capsulatus.

GENERAL SUMMARY.

1. In the course of experiments for obtaining pneumococci from the oral secretions of human individuals, the "plate method" described in this paper was found to be the most reliable method for isolating pneumococci.

2. An alkaline two-per-cent, glucose-serum-agar was regarded as the most favorable medium for the rapid development of colonies of the pneumococcus.

3. The morphological identification of the pneumococcus was made by means of a special capsule-stain. The recognition of the organism was accomplished with precision and reliability when present in the blood and exudate of test animals, and upon the various culture media employed.

4. Pure cultures of pneumococci could by the methods described be obtained within forty-eight hours for inoculation into animals for tests of virulence.

5. Normal persons often harbor the pneumococcus in their mouths. Of the seventy-eight cases examined, thirty-nine showed the presence of the pneumococcus.

6. In a second and larger series of studies on so-called normal cases by means of cultures made from the throat on Loeffler's medium, the organism was detected seventy-one times in 204 examinations, or in 34.8 %. In 145 cases the Streptococcus mucosus capsulatus occurred eight times (5.5 %), and the Friedländer bacillus twenty-one times (14.5 %).

7. Certain individuals may acquire the pneumococcus in their mouths in the hospital wards, and may continue to harbor it for a considerable period of time.

8. The pneumococcus may persist for days or weeks in the mouths of patients who have recovered from pneumonia.

9. From a study of patients in the hospital wards, certain conclusions in regard to communicability were drawn. "Normal" individuals, in whose mouths the pneumococcus is repeatedly found to be absent, may acquire the organism by association with pneumonia or "positive normal" cases. The handkerchiefs and dishes of pneumonia and "positive normal" cases are to be regarded as some of the means of transference of the organism from person to person. The lips of drinking-cups and the sputum or saliva in sputum cups were found to contain living and virulent pneumococci.

10. Certain characteristic morphological types of the pneumococcus were regularly met with in this study. These include the following: (1) the typical, (2) the small, (3) the large, (4) the bacillary, and (5) the streptococcus type of the pneumococcus.

11. Pneumococci in the mouths of normal individuals possess

the morphological and cultural properties which are characteristic of the same organisms when isolated from other sources.

12. All pneumococci possess the power of fermenting inulin with the formation of acid, even if not in all generations. But this holds true only when the inulin serum-water medium as modified by the writer is used.

13. A certain configuration of the colonies, designated by the term "ring-form," when present is diagnostic of the pneumococcus.

14. The percentage of virulent pneumococci present in the mouths of normal persons was 79; in cases of pneumonia, 77.

15. Pneumococci of the large mucoid type, and giving large mucoid colonies, should be distinguished from the Streptococcus mucosus capsulatus.

16. There are doubtful diplococci of atypical morphology which can be grouped neither with the pneumococci nor with the streptococci, although they possess many features in common with both.

17. The Streptococcus mucosus capsulatus belongs to a group which is related to, but distinct from, the pneumococcus.

18. Morphological characters and animal tests are of greatest value in the identification of the pneumococcus. By the employment of suitable culture media it is possible, in most instances, to recognize the pneumococcus by its morphology alone. The chief cultural tests are the following: the manner of growth upon the serum-glucose-agar, and the modified inulin-serum water, and the appearance of the "ring-type" of colony.

19. All pneumococci, irrespective of their source, were agglutinated by means of pneumococcus immune serum.

20. An immune pneumococcus serum was found capable of agglutinating various pyogenic streptococci, certain atypical organisms, and several strains of the Streptococcus mucosus capsulatus.

21. The serum of pneumonia patients varied in its power to agglutinate different pneumococci. Some strains were agglutinated, others not.

22. The sera of normal individuals and of normal rabbits

possess no agglutinating power for pneumococci, the atypical organisms, certain streptococci, and the Streptococcus mucosus capsulatus.

I wish to express my indebtedness to the attending staff of Mt. Sinai Hospital for their co-operation. Much of the material was taken from the wards of Drs. Rudisch, Meyer, Koplik, Brill, and Manges.

Further, I wish to thank Drs. Mandlebaum and Libman for many kindnesses, and Dr. Alfred Cohn, and Dr. Hertz, for their assistance.

A COMPARATIVE STUDY OF PNEUMOCOCCI AND ALLIED ORGANISMS.

The Report from the Central Laboratory to the Medical Commission for the Investigation of Acute Respiratory Diseases of the Department of Health of the City of New York.

By PHILIP HANSON HISS, Jr., M.D.,
Adjunct Professor of Bacteriology,

ASSISTED BY

John Harvey Borden, M.D., and Clinton Beecham Knapp, M.D.,
Alonzo Clark Scholar, *Assistant in Bacteriology and Hygiene,*
College of Physicians and Surgeons, Columbia University, New York.

GENERAL PART.

INTRODUCTORY.

The following paper contains the principal details and results of the work carried on, under the auspices of the Medical Commission for the Investigation of Acute Respiratory Diseases of the Department of Health of the City of New York, at the Bacteriological Laboratory of the College of Physicians and Surgeons, Columbia University.

In mapping out the bacteriological work of the Commission, it was deemed advisable to institute a central laboratory to which the cultures of pneumococci and allied organisms—particularly such cultures as seemed to show variations from the classic pneumococcus type—might be sent by the various workers for further study and identification. Such an arrangement, admitting of the study of the organisms under uniform cultural conditions and by one investigator or corps of investigators, would, it was hoped, not only prove a valuable biological study in itself, but possibly minimize errors of identification and thus increase the value of statistics ultimately to be based upon the findings in the series of cases investigated.

This work was undertaken by the writer, with the assistance of Dr. J. H. Borden and Dr. C. B. Knapp. The practical agglutination work was largely carried on by Dr. Borden, and the morphological and culture tests by Dr. Knapp.

For the information of the various workers under the Commission, and to obtain uniformity in the scheme of investigation, a comparatively full summary of the characters of pneumococci and streptococci, and the chief points to be determined in their recognition and differentiation, as well as suggestions as to the best methods of isolating them in pure culture and preserving their vitality, was sent from the central laboratory to each independent worker. The workers were also furnished with blanks [1]:

[1] INFORMATION TO BE OBTAINED ABOUT PERSONS FROM WHOM SPECIMENS ARE TAKEN.*

Case No. ..... *Date:* .....
Age: ..... *Sex:* ..... *Race:* .....
Residence: City..... Country .....
Occupation (in city or country):.....
Condition of Health:
Present State: Condition of naso-pharynx, mouth, bronchiand lungs, and middle ear?.....
.....
Past History: "Cold," catarrh, laryngitis, bronchitis, or any inflammation with which pneumococcus may have been associated?.....
.....
Is person subject to "colds," bronchial affections, or middle-ear trouble?....
.....
Does person use alcohol or tobacco? .....
Family History: Have members of family (mother, father, brother, or sister) suffered from an attack of pneumonia in past years?.....
.....
Association with Infected Individuals:
1. Has, or has had recently, any member of immediate family a "cold," catarrh, bronchitis, pneumonia, or any inflammatory condition with which pneumococcus may be associated (middle-ear trouble, mastoiditis, conjunctivitis)?
.....
2. Has person otherwise associated or come into contact with any one having a "cold," bronchitis, pneumonia, or middle-ear inflammation, mastoiditis, conjunctivitis?.....
.....
3. Does person often or with regularity ride in crowded tram, elevated, or steam cars?.....
.....
4. Does person frequent theatres or crowded shops or classrooms?.....
.....
5. Has person been exposed to poisonous gases or fumes, or other deleterious aërial agents?.....
.....
Subsequent History: Condition of person 15 days subsequent to examination. Has "cold," bronchitis, or pneumonia, or other pneumococcus infection developed?.....
.....
Negative Cases:
Complete clinical records should always be kept of all persons from whom specimens for examination are taken, whether organisms be obtained or not, in order to determine percentage of occurrence.
(Signature).....

* This blank is to be filled out, and sent to the Central Laboratory with the culture and its description.

one for recording the data concerning individuals from whom cultures were taken, and the other for recording the morphological, staining, and chief cultural characters and pathogenic action of the organisms isolated.

These blanks were to be filled out and returned with the cultures, for the information of the workers of the central laboratory.[2]

DESCRIPTION OF CHARACTERS CULTURE.*

Culture No. ..... *Case No.* ..... *Date of isolation:*.....
Source of Culture:.....
Saliva:..... Sputum:..... Throat and nose swab:.....
Technique of Isolation:
By Animal Inoculation..Rabbit or mouse died in..... days, ... hrs. with pure or mixed infection.
By Culture Method. Agar plate, pour or streak.....
.....
Morphology and Staining: (Gram's stain, Capsule stain, etc.)
In blood or exudate of original mouse or rabbit.....
.....
From culture media, agar, coagulated blood serum.....
.....
Were similar organisms determined in smears from original specimens?
.....
Cultural Characters:
Broth: .....
Agar: .....
.....
Loeffler's blood serum: .....
.....
Gelatin: Grows at ....° C. (24°, 22°, 18° C.).....
.....
Litmus milk: Acid in ..... days. Coagulated in ..... days.
Inulin serum-water: Coagulation does or does not take place in ..... days.
Pathogenicity: (Minimal fatal dose of suspension of 24-hr. agar culture in ..... c. c. 0.85 % salt solution).
How many generations removed from original isolation and on what media?
.....
Rabbit, weight ..... gms., dies in ..... days, ... hrs., from ..... c. c., subcutaneously, intravenously.
Mouse dies in ..... days, ... hrs., from ..... c. c. subcutaneously.
Agglutination:
Agglutinates at 1 to ..... in immune ..... serum. Control test with homologous culture shows an agglutination at 1 to ..... (Duration of observation, 3, 12, 24 hrs.) .....
Agglutinates in serum of person from whom culture was isolated at 1 to .....
Other observations and remarks:
(Signature).....

* This blank is to be filled out, and sent with cultures to Central Laboratory.

---

[2] It should be noted here that the work of each independent investigator, as recorded in his report to the Commission, represents his own findings and bacteriological identifications and not those of the workers of the central lab-

Object of the Bacteriological Study Instituted by the Commission.—As biological knowledge widens and new technical methods are discovered, confidence in identifications based on less complete studies and more primitive methods is necessarily shaken and statistics founded on these lose their value. It is only with a re-establishing of the identity of organisms in conformity with the new requirements, or by a determination of their dissimilarity, that the old statistics can again assume importance or may be definitely discarded.

This being the case, although much work had already been done by many investigators in connection with the bacterial flora of the normal mouth, and although no reasonable doubt existed as to the presence of the pneumococci in the mouths of a certain percentage of healthy individuals, still it was thought advisable to confirm, if possible, and, it might be, extend these statistics so far as practicable in the light of recent developments in our knowledge of the biology of pneumococci and streptococci, and to apply to the recognition and identification of these organisms the newer biological and technical methods. By such a study it seemed fair to suppose the true nature of the organisms from the mouths of healthy persons could be determined, and that their identity or non-identity with pneumococci derived from persons suffering from pneumonia and various pneumococcic infections could be definitely established.

Source of Cultures Examined.—The scope of the investigation included not only the examination of cultures isolated from the saliva and naso-pharynx of presumably healthy individuals, but also of organisms obtained from individuals suffering from "colds," pharyngitis, and bronchitis.

Cultures isolated from the sputum of pneumonia patients, or from their lungs at autopsy, as well as cultures from more truly

oratory. No systematic reports have been sent by the central laboratory to those sending cultures, and their reports have not therefore been influenced by the work of the central laboratory. Such a plan has the advantage of showing the percentage of discrepancy between the identifications of the same organisms by different workers, and the disadvantage, in connection with the present series of independent papers, in the interpretation of statistics based upon the findings.

internal sources, such as the circulating blood of pneumonia patients and other septicæmic cases, pleuritic exudates (empyæma), the spinal fluid in cerebro-spinal meningitis, abscesses, otitis media, or joint infections, were also investigated, so as to afford an extensive basis of comparison, and to determine the variations in morphology and biological characters that might occur among organisms of the pneumococcus type, which were found associated with pathologic lesions and definite infections.

Plan of Biological Study.—In undertaking the investigation of such an extensive series of organisms, it was realized that only the most promising lines of study could be followed in extenso at the central laboratory, and that many suggested methods of differentiation, as well as a detailed study of pathogenicity and the pathogenic effects could not be profitably undertaken. It was hoped that these studies would be carried out in detail by the other workers under the Commission, and the value of such suggested differential methods and pathogenic characters as a means of classification be thus determined.

The work of this laboratory has been chiefly confined to the determination of morphological characters and staining reactions, and of the growth characters presented by the organism on or in the usual culture media, and their fermentative activities in the presence of various carbohydrates and especially of inulin.

The immunization of animals against different strains of organisms and the careful study of the agglutinating action of the sera of such animals on the various organisms were also given much attention, and some of the most valuable results reached have been arrived at through these studies in agglutination.

SPECIAL PART.

The great number of organisms studied—in all about 260—precludes a detailed description of any but those representing the most important types or variations from these types. The most important details from the standpoint of differentiation and classification, however, are given in outline in a table at the end of the report.

MORPHOLOGY.

The morphology of the pneumococcus is in general—especially when repeated examinations are made—one of the most valuable guides to its identity.

When typical the pneumococcus is a rather large, lancet-shaped coccus occurring in pairs and surrounded by a definite and often wide capsule, which usually includes the two approximated cocci without a definite indentation opposite their line of division. The pneumococci may, however, occur singly, or in short chains, and even fairly long chains are not infrequently met with under artificial culture conditions. These may be chiefly due to the cultural conditions or be a prominent characteristic of certain strains. Apparently the capsules of organisms making up the chains are continuous; wavy indentations are usually present, however, in the capsule of chains, and at times distinct divisions are observed.

The chief variations from the typical morphology consist either in the assumption of a more distinctly spherical coccus type, or in an elongation approximating the bacillary form. Under certain conditions of artificial cultivation a distinct flattening of the organisms, particularly those making up chains, may be seen, and even the impression of the existence of a longitudinal line of division, characteristic of many streptococcus cultures, is not infrequently gained.

The capsules under certain conditions, especially in artificial media, may be absent or not demonstrable, and in certain strains capsules apparently may not be present under any conditions.

It is readily seen, therefore, that when the capsule is absent and the pneumococcus has at the same time assumed the spherical or flattened streptococcus type, identification by morphological examination is not possible. Even if the capsules are still intact, a definite identification of such streptococcus-like forms is not permissible, since encapsulated streptococci are not infrequently met with, which by cultural, fermentation, and agglutination tests are definitely separable from pneumococci.[3]

[3] See Hiss, *Ctbl. f. Bakt.*, 1902, xxxi, 302, and *Jour. Exp. Med.*, 1905, vii, 317.

In the opinion of the writer (Hiss), no classification based solely on morphology and the presence or absence of capsules, or the finer morphological variations of capsule formation, is of value. Practically any of the described variations may dominate one and the same culture under different or even apparently the same conditions of cultivation, and all grades may occur in capsule development from its typical formation, through all variations, to its total and apparently permanent absence.

Streptococci, as has been noted, may show capsule formation. This usually occurs, so as to be demonstrable by current capsule staining methods, only under certain and not well understood conditions, and the organisms tend to lose this character much more readily under artificial conditions than do the pneumococci. While some strains retain their power of forming capsules through many generations, the majority, under ordinary cultural conditions, usually have no demonstrable capsule.

Conditions Favoring Capsule Development and Demonstration.—The most favorable conditions known for the development of the pneumococcus capsules are found in the body fluids of man and animals suffering from pneumococcus infection. For instance, capsules may be demonstrated with ease by the usual methods in the blood, serum, and inflammatory exudates of the infected rabbit and white mouse, which are among test animals the most favorable for these experiments. Capsules may be equally well-marked in the fresh sputum of pneumonia patients, especially in the early stages of the disease, and in the exudates accompanying such pneumococcus infections as meningitis, otitis media, and empyæma. In sputum and the exudates of the various localized infections, the organisms are, however, frequently degenerated or under chemical conditions unfavorable for capsule staining, and satisfactory results are not then easily to be obtained.

The same is true of the scrapings from lungs of patients dead of pneumonia, often even in the state of red hepatization.

In artificial cultivation, if the nutrient medium is not milk or does not contain serum (serum, serum-agar, Loeffler's

coagulated blood-serum), capsules, by the ordinary methods of preparing and staining, are not usually to be demonstrated.

Capsules may, however, with much regularity be demonstrated on pneumococci from agar, broth, or almost if not all artificial media, irrespective of the length of time the organisms have been under artificial cultivation, if beef or rabbit serum is used as the diluent when they are spread on the cover-glass for staining. The copper sulphate or potassium carbonate method will then stain them readily.[4]

Capsules are usually not so readily demonstrated on streptococci, no matter what their source, and with no regularity, as compared with pneumococci. They may, however, often be demonstrated by the use of the potassium carbonate method, when the organisms have been grown in sugar-serum media or on ascitic-agar and then smeared on the cover-glass with serum. In some cultures the capsules are quite as sharp and well-defined as those of pneumococci, in others they are less well-marked, and in some appear as if in a semi-solid state and on the point of dissolving. Whether the capsules noticed on certain streptococci are of the same nature as that of the pneumococcus is at present open to doubt.

GENERAL CULTURAL CHARACTERS.

The growth characters on the usual culture media, as was to be supposed from our own previous work and the publications of other investigators, have afforded no practical basis for the differentiation of pneumococci from various simulating organisms and from streptococci. Routine tests were, however, made in neutral infusion-broth and gelatin, and on neutral infusion-agar and Loeffler's coagulated blood-serum, and in litmus milk. With the exception of gelatin and Loeffler's serum, no constant characters of diagnostic and differential worth were developed.

On Loeffler's coagulated blood-serum the pneumococcus usually develops into moist, rather watery discrete colonies which

[4] See Hiss, *ref. cit.*

tend to disappear after some days, while the streptococcus colonies, though also discrete, are usually drier and slightly whiter in appearance than those of the pneumococcus. The colonies of these two organisms, however, often so closely simulate each other that they cannot definitely be distinguished. The Loeffler serum has, however, been of value in aiding in the separation of the so-called Streptococcus mucosus from pneumococci. Streptococcus mucosus grows on this medium in a definite, smooth, watery layer, with fairly even edges, while the pneumococcus, as above noted, usually occurs in discrete watery colonies; and even when these colonies coalesce, there is not the same smoothness to the surface and edges of the growth as is shown by that of Streptococcus mucosus.

Gelatin has been useful in determining the limit of low-temperature growth of the cultures, and in identifying Streptococcus mucosus cultures.

Many cultures of pneumococci will not develop in gelatin at temperatures lower than 22° C. Others, however, even when freshly isolated, will grow fairly abundantly in gelatin at this temperature. This is true not only of typical organisms from pneumonic sputum and other pathologic sources, but equally so of organisms from the mouths of healthy persons, so that in these peculiarities of growth or non-growth at 22° C. or below, the mouth organisms have been found to agree, and in about the same proportion, with pneumococci from pathologic sources.

Streptococci and other organisms from the mouth, which are, so far as determinable at present, not pneumococci, usually grow readily and abundantly in gelatin at 22° C. or below. As their growth is generally closely similar to that of the pneumococcus in gelatin, this test is of little value in differential diagnosis. In the case, however, of Streptococcus mucosus the growth along the puncture in neutral infusion-gelatin, after some days' development at ordinary room temperatures, is readily to be distinguished from that of the pneumococcus and Streptococcus pyogenes. Instead of being a growth made up of fine discrete colonies representing the original line of inoculation, as in the case of pneumococci and of streptococci of various descriptions, the

growth of Streptococcus mucosus, especially in the deeper portions, where it seems to develop most abundantly and characteristically, is made up of larger globe-like colonies, which give one the impression that slight fluidification of the gelatin has taken place. This appearance is probably due to the large amount of capsular substance developed by this organism and is one of its most constant and distinctive characteristics. Cultures of this organism obtained by us from various sources, in the early days of our work, have continued to display this character and their typical growth on Loeffler's or other serum media, without the least noticeable change. This indicates that, no matter what the evolution and relationships of this organism may have been or are, the characters now distinguishing it from pneumococci are certainly in most instances of a markedly constant character, and that it is not apt rapidly to assume the characters of typical pneumococci on the one hand, or streptococci on the other. Observations indicating such a change may possibly be based on a mistake in primarily identifying a more or less confluent Loeffler's serum growth of a true pneumococcus with that of Streptococcus mucosus.

FERMENTATION TESTS—ACID PRODUCTION.

When we turn from the cultural characters as developed on and in the usual culture media—which are of so little aid in differentiating pneumococci from streptococci—to certain of the physiologic processes of these organisms, as indicated by their action on carbohydrates, the field of investigation is widened and decidedly more promising. In a series of investigations carried on by one of us (1) some four years ago, but only recently published in detail, it was shown that pneumococci have marked fermentative abilities, and that these are of wider scope than those possessed by the typical Streptococcus pyogenes and probably many other unclassified streptococci.

Pneumococci, it was shown, produced acid with ease from monosaccharids, disaccharids, and such complex saccharids as dextrin, glycogen, starch, and inulin. Streptococci, on the other hand, as represented by various supposed strains of the pyogenes

type, although producing acid with much regularity from some of these carbohydrates, usually did not have the ability to ferment starch and glycogen with the same regularity and ease as the pneumococci, and never, so far as the observations on about fifty cultures indicated, were able to produce acid from inulin. Inulin fermentation was therefore looked upon as a definite character of pneumococci, and probably as a valuable aid in differentiating atypical pneumococci from the non-inulin-fermenting Streptococcus pyogenes. Whether other species of streptococci or other lancet-shaped organisms simulating pneumococci possessed such an inulin-fermenting ability was not at that time determined.

The medium used as a basis for these fermentation tests was serum-water, composed of beef-serum one part and distilled water two or three parts, to which one per cent. of a five per cent. solution of highly purified litmus was added. The medium was then heated to 100° C. for a few moments and the various carbohydrates added in the proportion of one per cent., after which it was sterilized in the regular manner on three consecutive days.

In the present investigation, this same medium has been used as the nutrient base in the fermentation tests, which have included a study of all the organisms in dextrose, lactose, maltose, saccharose, raffinose, dextrin, glycogen, and inulin, as well as the alcohols mannit and dulcit.

A careful testing of the organisms sent to us by the various workers, as well as those isolated by ourselves during this work, has again demonstrated the inulin-fermenting power of pneumococci, and the value of this simple test in separating them from typical pyogenic streptococci as well as from various definitely encapsulated cocci which closely simulate the true pneumococcus. On the other hand, certain inulin-fermenting organisms have come to hand which we have not so far been able satisfactorily to identify either as pneumococci or streptococci of the pyogenes type. Some of these are definitely neither pneumococci nor streptococci of well-marked type, while others have a definite pneumococcus morphology but no capsule, or are

typically streptococci, the members of the latter being in general spherical and often occurring in long chains. These organisms for the most part lack capsules or only possess poorly developed ones. It is worthy of note that most of these atypical organisms were isolated either from the circulating blood of patients, or from some other internal source, or in many cases from pneumonic or other lungs at autopsy. The question of their probable identity will be considered more in detail in connection with the results obtained by agglutination tests in immune sera.

The chief point brought out by these fermentations, so far as its bearing on the immediate problem before the Commission is concerned, is that organisms morphologically of the pneumococcic type from the mouths of healthy individuals correspond absolutely in their fermentative characters with typical pneumococci from other sources, and thus a definite link is added to the chain of their connection with true pneumococci.

In studying organisms of the same or closely similar morphology, particularly such organisms as cannot be separated from each other by morphological or cultural characters of diagnostic value, two methods of great value have been developed during late years—fermentation tests, and the test for agglutination in immune sera. By these two methods differences in organisms have been discerned that were hardly suspected, or only guessed at by earlier investigators. In using such methods, however, which deal with the truly physiologic activities of organisms, care and patience must be expended upon prolonged observations and repeated tests. Due regard must be paid to the fact that transient and, it may be, permanent modifications may occur in the physiologic functions of organisms by exposure to unusual or adverse environment, and observations, therefore, based on short or superficial studies or tests of such organisms may be misleading. These changes are usually to be noted in the suppression or weakening of functions, upon which fermentations, agglutinations, and pathogenicity depend, and hardly extend to the acquisition of totally new functions, which probably are the product of a much more gradual and prolonged process of evolution and adaptation. These facts are well-

known, but attention is called to them here in connection with the interpretation of fermentation and agglutination results which, unless considered from this broad standpoint, are often confusing or lead to false conclusions. As an example, we may use the variations in the rate of fermentation of inulin by various cultures of pneumococci. A culture in full possession of this faculty may produce marked acid within from eighteen to twenty hours, while another culture, if tested only over five or six days, might be thought not to ferment inulin; after a longer time, or in repeated tests, however, this function will, so far as our experience goes, eventually reveal itself. Variations in the rate of fermentation are often marked in the same culture, differences of days in the time required for complete fermentation often being noted.

In the absence of all information as to the reaction of organisms in specific immune sera, fermentations seem, therefore, the safest guide, other things being equal, to the identification of organisms. Where fermentations have been carefully studied and the identity of organisms thus determined, one can usually predict with much certainty what the results of tests in immune sera will be. However, it must be remembered that variations in agglutinative functions also occur and may be fairly permanent, thus leading to false conclusions, unless, on the other hand, due regard be paid to the other biological characters as means of identification, and to the source of the culture. Changes in the limit of agglutination of undoubtedly the same species or even the same culture, after exposure to certain environments, are well known to those familiar with typhoid agglutinations, and in considering, in the following section, some of our agglutination results, the same phenomenon will be noticed to occur among the pneumococci.

AGGLUTINATION TESTS.[5]

In undertaking the systematic comparative study of the agglutinations of pneumococci and allied organisms from various

[5] The table has been placed at the end of the paper.

sources, the workers under the Commission had to enter a practically uncultivated field of research. No guiding statistics of value existed and the technical procedures, at least those available for such an extensive study, had not been worked out. The same statement is equally true of streptococcus agglutinations and agglutination technique. Agglutination tests with both pneumococci and streptococci, where the usual broth cultures (either with or without sugar added) or emulsions from agar had been employed, had in the experience of most workers been found not only technically unsatisfactory, but had given varying and often contradictory results.

After a few trials of the older methods it was obvious, therefore, if advances were to be made in our knowledge of the agglutinations of these organisms, that a new as well as a simple and easily handled technique had, if possible, to be devised.

The first problem was to get the organisms in a proper and dense enough emulsion for observation of agglutination, and the second to obtain them in the biological state in which they would respond definitely and with regularity to the action of the agglutinins, for with the old procedures, as stated, only irregular and unsatisfactory agglutinations had been obtained. The only techinque already in use which in any degree conformed to these conditions was that recommended by Wadsworth.[6] This is valuable and reliable, but when a long series of organisms, as in this comparative study, have to be tested against the same or various sera, almost a prohibitive amount of time and energy must be consumed, when following this technique, in centrifugating and preparing the organisms for the tests.

A method which met the requirements was finally worked out by us. For purposes of reference it is given here in the words of the original paper which has but recently been published (2):

"The method gives results and appearances which are entirely comparable to those familiar in typhoid, dysentery, and various other agglutinations, and is simple and particularly available for the study of pneumococci and streptococci and other organisms which ferment carbohydrates, but which thrive poorly or die out rapidly in the usual media. The streptococci often, as is well

[6] Wadsworth, *Journ. Med. Research*, 1903, x, 228.

known, grow in broth, either with or without sugar, in flocculi or thickly matted masses entirely useless for agglutination purposes. By the proposed method usually a fair and often a good and satisfactory emulsion may be obtained, from which agglutinative limits may easily be determined.

"The medium used should be a one- or two-per-cent. peptone broth made from meat infusion, which has been brought to neutrality before boiling and coagulation. After filtration for clearing, one per cent. of dextrose (or other sugar fermentable by the given organism) and one per cent. of calcium carbonate are added. If the medium be acid, the latter salt will of course bring it to neutral. The calcium carbonate may then be well distributed throughout the broth by shaking and the emulsion rapidly decanted into tubes or preferably small Erlenmeyer or Florence flasks, say 100 cubic centimeters to 150 cubic centimeters in each. These are sterilized on three consecutive days at 100° C. in the usual manner. The flasks after inoculation are placed at 37° C. and are thoroughly shaken once or twice a day to neutralize the acid formed and to break up the chains and masses in the case of streptococci. The growth may be sufficient for purposes of agglutination in two days or even in one day, but as a routine up to the present time we have employed a three or four days' growth, which seems to give more uniform results and more marked agglutination.[7]

"About an hour before using for agglutination tests the culture should be thoroughly shaken and the calcium carbonate and larger clumps, if present, allowed to settle during this time. The sample to be tested should then be taken from the upper portion of the fluid; or the cultures, after shaking, may be centrifugated for a few minutes. This centrifugalization, however, is not necessary if one remembers that a slight primary deposit may occur which is not due to agglutination. . . .

"Routine agglutination tests are made by adding one cubic centimeter of the serum dilution to one cubic centimeter of the emulsion in small test-tubes. The tubes are placed at 37° C. for two or three hours—after which time the agglutinations are often practically complete—and then transferred to the ice-box to prevent growth taking place and permit of the further deposition of the clumps of agglutinated organisms, and the final control reading made after eighteen to twenty-four hours. . . .

"Spontaneously agglutinating cultures, of course, are met with among the pneumococci, and are frequent with streptococci, but even with these the limits of agglutination can be determined with much certainty if careful comparison with the control is made."

In the course of our work many hundreds of serial tests have been made by this method, and it has proved of great value not only in the ease of application but in the comparative uniformity of results given by it. Readings up to eight hundred and over

[7] Such cultures may then be preserved in the ice-box. We have tested them frequently in the same sera from day to day, and have found little or no change in their limit of agglutination even after weeks.

are not rare in the sera of rabbits immunized to pneumococci, and the serum of streptococcus immune rabbits has in some instances agglutinated the homologous streptococcus cultures in dilutions over 6400. Reported agglutinations of pneumococci made by the usual method rarely indicate an agglutinating power of the sera over 100, and such readings are obtained with no uniformity. Our success in getting such high agglutinations has probably not only been due to our method of making the test, but in part, at least, to the use for inoculation of the animals of mass cultures obtained by growing the organism for this purpose in the calcium-carbonate-glucose broth. Our animals, although usually very gradually immunized at first, eventually received large doses of these growths intravenously, and for the most part survived them and remained in good condition. Rabbits, however, immunized against Streptococcus mucosus in several instances became much emaciated and eventually died.

A series of animals was immunized with pneumococci from pneumonic sputum, and from the mouths of healthy individuals, also with Streptococcus pyogenes, Streptococcus mucosus, and various other organisms of peculiar types. The sera obtained from these animals and from normal rabbits afforded the basis for an extended study of the agglutination reactions of the various cultures.

All the organisms of our series have been tested against the immune pneumococcus sera, and their agglutinations controlled by tests in normal rabbit serum and normal salt solution, and a careful comparison made with the results given by the homologous immunizing cultures.[8]

Pneumococci as a rule, no matter what their source, do not agglutinate in high dilutions of normal rabbit serum, rarely over 1:10 to 1:20, nor have they been found to agglutinate to any

[8] There may be a slight false primary clumping in all tubes of the series, including the controls. These clumps are apt to disappear later. Even if they persist they do not settle out rapidly, and the specific agglutination is readily distinguished from them. Such occurrences, however, should make one exceedingly cautious in accepting results reported in pneumococcus and streptococcus agglutinations when the hanging-drop, microscopic-test method alone has been employed.

marked extent in streptococcus immune serum or, with the exception of Streptococcus mucosus serum, in the immune serum obtained by inoculation with other organisms of various types.

Nearly without exception, however, organisms previously recognized by morphological, staining, and fermentation tests as distinctly of the pneumococcus type, have been found to agglutinate in pneumococcus immune serum, and the results obtained, taking the series of these organisms as a whole, have shown remarkable uniformity in degree of agglutination and a close approximation to that given by the homologous organism. This is true not only of pneumococci from recognized pathologic sources, such as pneumonic sputum, etc., but of the organisms of pneumococcic type from the mouths of supposedly healthy persons. The same results are obtained in the sera of animals immunized against these latter as in the sera of those immunized against pneumococci from pathologic sources. In other words, the agglutination tests have fully confirmed the complete identity, which was presaged by the fermentation tests, of organisms of pneumococcic type, from the mouths of healthy individuals, with those from pathologic sources. Further than this, these tests have also reinforced the evidence given by fermentation tests, namely, that there are distinctly encapsulated, Gram-positive organisms which may be met with and which simulate pneumococci too closely for morphological separation, but which are, nevertheless, according to agglutination and fermentation tests, separate and distinct from pneumococci. These remarks do not apply to Streptococcus mucosus, which is peculiar in its agglutination reactions, showing only moderate agglutination in the sera of animals, which according to all standards are highly immunized against it. Agglutinations of 1:20 to 1:50 generally abruptly mark the limit. In pneumococcus immune serum it shows little or no agglutination. On the other hand, pneumococci agglutinate in mucosus immune serum in very high dilutions. From this fact and the fact that the fermentations caused by these two organisms are, so far as we know, coextensive, we have been practically forced to the conclusion that Streptococcus mucosus is not a distinct species, but a variety of

pneumococcus, which is, however, very firmly established in the possession of certain morphological peculiarities, especially as relates to the abundant production of mucinous or capsular material. Its peculiarities in agglutination, or rather non-agglutination, may be, we have thought, closely connected with the over-production of this special mucous or mucinous material and its solution in the culture fluids—in other words, that this material may have a combining or inhibitive action on the agglutinins. In cultures centrifugated and washed in salt solution we have, however, failed to increase markedly its agglutination, though some slight increase did take place. What the explanation is we at present do not know, but the fact that pneumococci agglutinate to such a marked degree in Streptococcus mucosus immune serum argues a close relationship between the two.

IMMUNE SERA.

	Pneum. "1."	Pneum. "3."	Pneum. "23."	S. muc. "7."	S. muc. "7-a."	S. pyog. "1."
Pn. "1".....	*400–800*	200–800	400–800	200–800	400	0–100
" "3".....		*400–800*			200–800	0–100
" "23".....		100–800	*200–800*	100–200		
" "45".....		400–800	100–200	200–800	100–400	
" "E. 1" ...		100–800			100–200	
" "E. 32" ...		200–800		100+		
" "E. 55" ...	100–400		400–800	200–400		
" "N. 7" ...	200–800		800	200–800	200–800	
" "N. 17" ...	200–800		800	200–800	200–800	
S. pyog. "1".....		200–800				*800–6400*
S. muc. "7".....		0–10		*10–100*	*10–200*	0–50
" "22"		0–10		10–50	0–50	—
" "30".....		0–20		10–20	0–20	0–20
" "55. a".....	0–10			10–50		
" "Br.".....	0–10			10–50		

The immunizing organisms Pn. "1" and Pn. "3" are from pneumonic sputum: Pn. "23" is from the normal mouth; and the other cultures from normal mouths and various pathologic sources.

The italics indicate the agglutination in the homologous serum.

When two numbers are used to record the results of a test, the first indicates the last dilution in which full precipitation of the agglutinated organisms occurred, and the second the highest reading with a hand lens.

The results of control tests in normal rabbit serum are not recorded in the table. A few of the pneumococcus cultures showed a very slight agglutination at 1:10. The Streptococcus pyogenes culture agglutinated at 1:100. None of the Streptococcus mucosus cultures showed agglutinations at 1:10.

The above table illustrates the agglutination of pneumococci

in pneumococcus and Streptococcus mucosus immune sera, as well as in Streptococcus pyogenes immune serum and normal rabbit serum. The agglutinations of Streptococcus mucosus and Streptococcus pyogenes are included in the table for comparison.

Before leaving the subject of the agglutination of pneumococci, which are typical morphologically as well as in their fermentation reaction, it must be noted that certain of them when compared with the homologous organisms in immune serum show a very low grade of agglutination. A glance at the full table at the end of the report will show that such organisms are usually not from the normal mouth or from pneumonic sputum, but from some more internal source, such as the blood, or some chronic and deep-seated lesion, or lungs at autopsy. These results are in line with those found in the case of typhoid bacilli from the circulation and from more chronic lesions, as well as those grown artificially in immune serum, and are probably an illustration of the modification of function by environment.

Of the organisms examined by us which do not ferment inulin and which are of typical streptococcus morphology, or even of diplococcus type and slightly lancet-shaped but non-capsulated, little need be said here. The results of their agglutination tests will be found in the appended table. It is simply worthy of note that some streptococci show marked agglutination in normal rabbit serum and naturally, therefore, also in anti-pneumococcic serum, and that unless controls be made false conclusions might be drawn from this. In their homologous sera they may agglutinate in high dilutions. Another point made clear is that various streptococci of the same morphological and possibly even the same fermentative and cultural characters do not, with anything like the uniformity displayed by pneumococcus cultures, agglutinate in a given streptococcus serum. This indicates the possibility of a future satisfactory classification on this basis.

The other organisms of the series, which have not as yet been mentioned but which deserve especial attention, are inulin-fermenters of pneumococcus morphology but without capsules, other lancet organisms not so definitely of the pneumococcus

type, and organisms apparently of definite streptococcus morphology, all of which ferment inulin.

Some of the first variety, if not all, are undoubtedly pneumococci, and agglutinate in pneumococcus immune serum, though usually not in high dilutions, and there seems no reason for placing them in a separate class.

Other inulin fermenters, which are small organisms, at times showing lancet morphology, but which are usually non-capsulated, and vary in some of the less important features in artificial media, it is difficult to classify, as they show little or no agglutination in pneumococcus serum. Whether they are modified pneumococci or should be placed in a distinct class, our studies at the present writing have failed to determine. Their agglutinations are recorded in the table. Apparently all of them are not of the same type. If the requisite time had been at our disposal, a special study of the pathogenic effects of these organisms on the one hand, and on the other of the modifying influence on the organisms themselves of a residence in the animal body, would have been undertaken.

Our attention has, however, been especially directed to certain non-capsulated, inulin-fermenting cultures kindly sent to us by Dr. Charles Norris and Dr. Leo Buerger. Two of the cultures from Dr. Norris show variations from the classic pneumococcus type. One of these, known by us as "Nor. 199," only varies from this type in not possessing a capsule. Its morphology otherwise is typical, and its growth in rabbit blood-agar cultivations corresponds absolutely to the growth given by the vast majority of pneumococci in this medium. It agglutinates to some extent in pneumococcus immune serum and is probably a true pneumococcus.

The other culture, "Nor. 102," has no capsules and shows a less typical lancet morphology. Its growth in blood-agar plates is not that of the most diagnostic type, but corresponds to a type seen, nevertheless, among certain otherwise perfectly typical pneumococci. No agglutination in pneumococcus serum has been shown. The weight of evidence is, however, in favor of its being a temporarily or permanently modified pneumococcus.

Dr. Buerger's culture, known as "Streptococcus No. 7," ferments inulin. Its morphology is in general of streptococcus type, but lancet forms at times may predominate. Very narrow capsules have been observed by me on some cultivations of this organism. This organism does not agglutinate to an appreciable degree, according to our macroscopic tube agglutinations, in anti-pneumococcus sera, but gives high agglutination in its homologous serum. Some cultures of pneumococci agglutinate in this serum even in high dilutions. In blood-agar plates the colonies appear brown or dark-green surrounded by an opaque area—the whole plate assuming a distinct greenish tinge, which is more marked, if anything, than the tinge occurring in most pneumococcus plates.

The organism is definitely not a streptococcus of the pyogenes type, and probably not a true streptococcus, unless it is of the type described by Schottmüller as Streptococcus mitior viridans. Not having had the opportunity of examining Schottmüller's original cultures, it is, of course, not known to us whether they ferment inulin or are non-inulin fermenters of streptococcus type, such as have been met with in our present studies, and which also produce a greenish color but no clear lysis in blood-agar plates. The true status of this Buerger streptococcus (?) we have, therefore, up to the present been unable satisfactorily to determine, though it may be an atypical pneumococcus.

GROWTH CHARACTERS IN BLOOD-AGAR.

Schottmüller (3) in 1903 and, independently of him, Rosenow (4) in 1904 called attention to certain reactions caused by pneumococci and streptococci when growing in agar with which human or animal blood had been mixed.

Schottmüller stated that pneumococcus colonies developing in this medium usually became of a greenish tinge, and were surrounded by a zone of opacity of a greenish color. Streptococci of the erysipelatos type did not assume a dark or greenish tinge, and were surrounded by a distinct clear zone due to complete lysis of the red corpuscles and change of the hæmoglobin. Rosenow's observations confirmed these.

Schottmüller further described a form of streptococcus which he designated Streptococcus mitior viridans. This was less virulent than the other type and was said to be usually associated with rather chronic lesions and septicæmias. It was described as of streptococcus morphology and non-capsulated. In blood-agar plates it simulated the pneumococcus, but the greenish tinge was less intense. Little or no hæmolysis occurred. What the true nature of these organisms is, is in doubt, as nothing is known of their fermentations and agglutinations.

In our series of organisms we have found the action of pneumococci, as a rule, to conform to Schottmüller's description, but usually with a deep-brown or reddish tinge to the colonies rather than a definite green, the opaque area of partial hæmolysis (and precipitation (?)), but not clearing, and of yellowish or greenish tinge being present.

Some organisms of our series of true non-inulin-fermenting streptococcus type conformed to his description of Streptococcus erysipelatos with the clear zone surrounding their colonies and no greenish tinge. Other non-inulin-fermenting organisms of general streptococcus type, some capsulated (see P. and S., No. 8) and some not capsulated, gave the typical pneumococcus or Streptococcus mitior pictures, which are really not to be distinguished from each other.

Our unclassified inulin-fermenting organisms either produced no lysis or change in color of the medium, or gave rise to appearances closely suggesting or absolutely corresponding to those given by pneumococci.

Whether this can be depended upon as indicating their pneumococcus nature, one would hesitate to say in face of the fact that so many non-inulin-fermenting, non-agglutinating, and definitely not pneumococcus cultures also give rise to these appearances.

In streak cultures on slanted rabbit-blood-agar in tubes,[9] the writer, in making a comparative observation on about two hun-

[9] This medium is made by bleeding a rabbit from the carotid artery, through a sterile canula and rubber tube, directly into tubes of fluid sterile agar (kept at from 45° to 50° C.). About one-half to one cubic centimeter of blood should be added to each tube. The blood must be mixed immediately with the agar (to prevent clotting), and the tubes slanted and allowed to harden.

dred pneumococci, streptococci, and miscellaneous unidentified organisms, noted certain appearances which, when prominent, seemed to be practically diagnostic of the pneumococcus cultures.

Streak cultures of pneumococci on mixed blood-agar grow at first with the usual characters seen on serum media. The growth, however, appears usually of a rather dirty yellowish tinge, and the blood-agar in immediate contact with the growth and at times for some distance from it takes on a yellowish rather opaque look, due to decolorization and probably to a slight precipitation from acid formation. The variations met with are usually in the direction of a less typical growth layer, combined with more decolorization and hæmolysis, though the medium remains opaque.

During the first few days of growth neither of these appearances is characteristic When, however, the tubes have remained in the incubator some days, the majority of the pneumococcus cultures take on a distinctly characteristic appearance. Most of the growth disappears, leaving the general decolorized dirty-yellowish area, while definite raised colony-like nodular masses remain, which usually are of a brownish-black or dark-red color, as if the masses had become distinctly stained with the blood pigments. One gets the impression of a blistered painted surface. None of the streptococci or other organisms, so far as determined by this test, assumed this appearance. Whenever this appearance was noted, the organisms were found to be true pneumococci. The cultures of pneumococci, giving less typical growth and more extensive decolorization of the medium, retained the more even appearance of surface and did not become nodular. So far as we know, these cultures are true pneumococci, but, it may possibly be that they are modified Streptococcus mucosus cultures which had not previously been recognized. The Streptococcus mucosus growth, though at first prominent and moist and quite characteristic on this medium, soon practically disappears. Traces of decolorization may or may not be prominent. One culture, however, presented an appearance similar to that of the pneumococcus.

PATHOGENICITY.

This phase of study has not, as was stated earlier, been taken up with any regularity in our comparative study. Simple tests of the pathogenicity of organisms, especially such organisms as pneumococci and streptococci, usually give little information of value in classification, and even definite studies of lesions caused by such organisms are not often of diagnostic aid.

It has been generally demonstrated by all investigators that pneumococci as a class, especially when freshly isolated, are usually pathogenic, although in varying degrees, for white mice and rabbits; while streptococci, even from severe lesions in man, may show little or no pathogenicity for mice and rabbits and other test animals. Both of these organisms, even when primarily virulent in high degree, tend to lose this character when cultivated on the usual artificial media. Any tests, therefore, to determine the pathogenicity of the organisms, when they arrived at our laboratory, would have led to little or no information of value as to the original pathogenicity of the cultures. This work was left in the hands of the investigators who made the isolations.

All that need be said here is that so far as our own cultures are concerned and so far as we can gather from the information sent to us by the workers in other places, little or no difference has been determined in the relative pathogenicity of organisms of pneumococcic type from the mouths of healthy individuals and those from persons suffering from pneumococcic infections.

If such pathogenicity for animals indicates in any way the grade of pathogenicity of organisms for man,—a supposition always open to grave doubt,—and if, especially, these organisms be found to linger in normal mouths during the summer months, the possession of virulency by organisms from healthy individuals is a matter of interest and importance in the consideration of the mouth as a nidus of these infective agents and the possible and probable ways of their preservation and dissemination.

REMARKS ON THE CLASSIFICATION OF THE ORGANISMS INVESTIGATED.

An attempt, at this stage of our work, to classify definitely all the organisms which have been received by us would be ill-advised. Many of these have but recently come into our hands, and when not responding definitely to the tests and showing easily recognized and diagnostic characters of some one of the well-known types, our acquaintance with them has been too limited to warrant an attempt at classification. Any points, however, so far determined about their general morphology and biology, are recorded in the table.

The following tentative classification is given for purposes of reference and as an illustration of some of the types met with:

A. Inulin fermenters.

1. Typical pneumococcus morphology. Typical capsules. Agglutinate in pneumococcus immune serum. Growth on or in blood-agar usually typical. *Typical pneumococci.*
2. Typical pneumococcus morphology. No capsules determined, even in body fluids of infected animals. Agglutinate in pneumococcus immune serum but usually not in very high dilutions. Growth in blood-agar same as that of typical pneumococci. *Pneumococci.*
3. Atypical morphology. Lancet-shaped organisms occur, but morphology approximates streptococcus type, or some cultures may be composed of small lancet-shaped diplococci. Capsules, usually not well-marked, may be present. Growth in blood-agar plates may or may not be typical. Do not show nodular growth on blood-agar streak cultivations. Organisms mostly from pneumonic lungs at autopsy or from some internal body source. Do not ordinarily show diagnostic agglutinations in pneumococcus immune serum, but may agglutinate in homologous sera to fair degree. Pneumococci do not, as a rule, agglutinate diagnostically in these sera. Probably a mixed group, some of which are presumably true but atypical pneumococci, or atypical Streptococcus mucosus.
4. Morphology practically like that of the pneumococcus,

but chains are more frequent and the elements are more usually spherical. Capsules typical and well-marked. Agglutinates only in low dilutions of homologous immune serum or in pneumococcus immune serum. Pneumococci agglutinate in high dilutions of the serum of animals inoculated with this type. Separated from pneumococci by peculiarities of growth in gelatin and on Loeffler's and other serum media. *Typical Streptococcus mucosus;* probably a variety of pneumococcus.

B. Non-inulin fermenters.

5. Capsulated organisms simulating pneumococci, but of general streptococcus morphology. Capsules fairly constant on artificial media. Ferment many sugars, but not inulin. Simulate pneumococcus growth and reactions in blood-agar plates, but do not show nodular growth on blood-agar streak cultivations. Do not agglutinate in pneumococcus immune serum. Agglutinate in moderate dilutions of homologous serum, in which serum the pneumococcus does not agglutinate. *Streptococcus mitior viridans* (?).
6. True streptococcus morphology. Ordinarily capsules are not demonstrable. Ferment many sugars but never inulin. Colonies in blood-agar plates not green-tinged, but surrounded usually by a well-marked clear zone. Apt to agglutinate in normal rabbit serum, even up to 1 : 200. Agglutinate markedly in homologous sera. Pneumococci are practically not agglutinated by these sera. *Typical Streptococcus pyogenes* (*erysipelatos*).
7. Streptococcus morphology. May or may not have capsule. Ferment many sugars. Orange growth along puncture in Welch's and other media. Agglutinations in general not determined, but do not agglutinate in pneumococcus serum. May be mixed group.
8. Streptococcus morphology. May or may not have capsules. Sugars not fermented, with possible exception of some monosaccharids. Do not agglutinate in pneumococcus immune serum.

It should be noted in connection with this classification of the streptococci, that it is merely in outline, and that other classifications, possibly of value, are to be arrived at from a detailed grouping by fermentation reactions. Attention was called to this subject by the writer in a previous paper. A close study of agglutination, taken in connection with the groupings suggested by fermentation reactions, will probably lead to a separation of streptococci into distinct and easily recognizable groups.

So far as the whole classification is concerned, it should again be recalled that variations of a fairly permanent or even of a transient nature in the morphology and physiology of organisms may definitely interfere with their recognition, and when classifications have to be based on such delicate biological processes as are involved in fermentations and agglutinations, only long-extended and painstaking observations will lead to an eventual recognition of their true identity.

In connection with this statement and the following remarks upon statistics, it is of value to note the percentage of discrepancies between the results obtained by us from our examination of the cultures sent to the central laboratory and the results which were obtained by the various workers from their examination of these same cultures and reported to us. Out of 146 instances in which the cultures were reported as fermenting inulin, we have only been able to determine the occurrence of such fermentation in the case of 119 of the cultures, a difference of 18½ per cent. The results of our inulin determinations were, nearly without exception, supported by the agglutination tests —in only two instances, in fact, where inulin fermentation was reported and our tests gave negative results did the agglutination results seem to indicate the pneumococcus nature of the organisms. In both of these cultures the organisms had a distinct streptococcus morphology, and as streptococci frequently agglutinate in high dilutions of normal and heterologous sera these results are not determinative.

In only one instance did we find inulin fermentation when none was reported, and in this culture the morphology was perfectly typical, and typical capsules were present. Our

results show in this instance, however, that no agglutination took place in pneumococcus immune serum.

This discrepancy of nearly twenty per cent., indicating as it does the differences in identifications of the same organisms when studied by different investigators, has an extremely important bearing upon the percentage of error which in the ordinary course of events must be allowed in the consideration of statistics from various sources.

STATISTICAL.

Bacteria of Pneumococcus Type in Normal Mouths.—Enough has been said in the previous part of this paper to make it clear that there occur in the mouths of healthy persons organisms that show, according to the most refined morphological and biological tests, all the characters displayed by true pneumococci from pathological sources. No reasonable doubt can now exist as to their true pneumococcus character.

One of the fundamental objects of the bacteriological work instituted by the Commission—the solution of the problem of the nature of these organisms—may therefore be looked upon as accomplished.

Allied to this a point of great importance has also, we think, been conclusively demonstrated, namely, other organisms are to be met with which too closely simulate pneumococci morphologically to be separated from them by the most careful morphological examination or by the usual routine cultural tests, only fermentation and agglutination tests definitely establishing their nature. The futility of attempting to base statistics on purely morphological findings and staining reactions, or even superficial culture tests, must, therefore, be obvious. At the best they are but indicative.[10]

Another object of the Commission, necessarily secondary to the establishing of the nature of mouth organisms, was the de-

[10] Old statistical statements as to the frequency of occurrence of pneumococci in normal mouths, although this frequency may be found to approximate closely present findings, must, nevertheless, in the light of these facts, lose any just claim to scientific accuracy.

termination of the frequency of occurrence of pneumococci in the mouths of healthy individuals.

For such studies to be of definite statistical value it was obvious that series of individuals living under different environmental conditions should be examined. These were indicated as follows:

1. *Normal persons*, giving history of no known contact with a source of infection and no recent history of "cold," bronchitis, or pneumonia, middle ear, conjunctival, or other possible pneumococcic infection.

2. *Normal persons*, intimately associated with patients suffering from pneumococcic infections—i.e., pneumonia, bronchitis, etc. (nurses, doctors, hospital attendants, and non-pneumonic patients in wards with pneumonia patients).

These investigations were not to be confined to one locality, but to be carried on in other places besides New York—in particular in Philadelphia, Boston, and Chicago,—thus assuming a much more general significance, and in themselves affording a basis of comparison of frequency of occurrence in different localities. The results of this investigation by the various workers will doubtless eventually be correlated and tabulated; at present they are scattered throughout the independent reports.

Before considering the results of our own investigations along these lines, attention should be called to certain factors that may influence deductions made from such findings. Considered from the standpoint of a simple illustration of the ease of general dissemination of organisms thrown off from the bodies of diseased persons by way of the mouth and nose, the finding of pneumococci in the mouths of normal individuals may seem to have great significance. Another interpretation is, however, perfectly legitimate from such simple data; in other words, these organisms may be common and permanent inhabitants of mouths, as colon bacilli are supposed to be of the digestive tract. If such a view were substantiated, the application of protective hygienic measures might assume less importance.

There seem to be two definite ways of solving this problem.

Of these the only one open to the investigator in cities is the careful examination over extended periods of the bacterial flora of the mouth of one and the same individual. In this way the permanent flora of such a mouth may soon be determined and the appearance or disappearance of organisms noted with accuracy. Even varieties of organisms of the same species may be recognized, when thoroughly investigated, by some minor biological or possibly morphological peculiarity, and thus definitely distinguished from a new invader of the same species. If, then, upon the advent of such an invader pathologic changes occurred, its association with these would be fairly well established, and the entire period of its residence could be determined. The other method of solving this problem is the investigation of individuals living in localities where pneumonia does not ordinarily occur, or of individuals not coming frequently into contact with large masses of population. As a possible means of arriving at a solution of this problem, the writer has suggested the examination of the mouths of such persons as sailors arriving from long voyages by sea.[11]

By such methods as these the permanency of the occupation of the normal mouth by such organisms could be determined, or their definitely transient nature be shown. This will be referred to again in considering the statistics of our own investigations.

Our own investigations on the bacterial flora of the mouths of normal persons was limited to a study of the saliva from twenty-two persons not known to be suffering from any form of pneumococcic infection. In the case of fifteen of these individuals only one examination was made, and this in each instance by mouse inoculation. If the mouse did not die within a reasonable time the bacteria of the local lesions were investigated. Organisms of definite pneumococcus type were isolated from seven of the fifteen persons, i.e., in 46.6 %. The saliva from the remaining seven persons was tested repeatedly, and pneumococci were demonstrated in the saliva of six out of the seven, i.e., in 85.7 %.

[11] This work is now being carried on at the New York Quarantine Station, and, it is hoped, may throw some light upon the subject of the persistence of pneumococci in normal mouths.

These higher figures may be interpreted in two ways, either as representing a correction of errors due to the single application of any given method of isolating organisms, or to this in part and in part to the detection of new invaders, since the examinations were made at intervals, over several months. No matter what the actual interpretation, the prominent fact brought out is that practically every individual, at least during the winter season when exposed to environmental conditions such as those existing in New York City, acts as host, at some time or other, and probably at repeated intervals, for organisms of the most characteristic pneumococcus type. In answer to the question above proposed as to the continuous residence of such organisms as parasites of the mouths of normal persons, it may be said that it is the writer's opinion that in the majority of cases the residence of any given strain of pneumococci under such conditions is apt to be temporary rather than permanent or long continued.

Some of the reasons for such an opinion may be given: in the first place, repeated examinations carried on by all methods at short intervals may fail for a longer or shorter time to demonstrate the presence of pneumococci in the mouths of a certain percentage of normal individuals. This, taken in connection with their practically universal occurrence, indicates that organisms of this type tend to disappear from such mouths; and furthermore, even when pneumococci are found repeatedly and constantly throughout long periods, unless the strain present has some minor peculiarity by which it may be recognized from new invaders, we cannot argue that we are not dealing with newly acquired organisms at each examination.

In the second place, unless such sensitive organisms rapidly become adapted to some more or less protected nidus in the mouth or naso-pharynx, such as the tonsillar crypts or, as it seems not unlikely to the writer, to a parasitic residence in the salivary ducts, the ordinary conditions in the mouth during the taking of foods, especially of an acid nature, and the rapid osmotic changes to which the organisms in general must be subjected when food or drink is in the mouth, seem highly unfavorable for organisms so sensitive to their environment as

pneumococci undoubtedly are under observed cultural conditions. Further than this, flora already adapted may interfere with the occupation by these new forms. It seems not unlikely, therefore, that such invaders usually, unless particularly favorable conditions (temporary or permanent) for their adaptation to this new environment exist, would tend to die off after a comparatively short residence.

PNEUMOCOCCI IN THEIR RELATION TO "COLDS."

When the repeated investigation of the flora of the same mouth was taken up, we had in view not only the determination of the normal and transient bacterial inhabitants, but also of the relation of these latter to disease processes which might manifest themselves during the period of such examinations. In most instances, so far as the histories showed, no "cold," pharyngitis, bronchitis, or pneumonia, either preceded, within a recent period, the determination of pneumococci in the mouth, or developed subsequent to the invasion of the mouth or nasopharynx by these organisms. In two instances, however, the appearance and residence of pneumococci in the mouths of persons hitherto free from them were incident with the development and course of "colds." Subsequent examinations in one case showed the early disappearance of the organisms; in the other, in which the inflammatory process had been much more severe and a purulent post-nasal discharge was developed, the organism (presumably the same) had remained in the mouth up to the last examination, about a month subsequent to the cessation of all symptoms of infection. In the purulent discharge pneumococci were present in practically pure culture and in the greatest profusion.

In each of these cases, it is interesting to note as an illustration of the value of careful study in recognizing organisms, that the mouth seemed to be the permanent habitat of organisms of general pneumococcus morphology—in one instance of a Gram-positive, capsulated, non-inulin-fermenting streptococcus morphologically not definitely to be distinguished from the pneumococcus; in the other, of a typical Streptococcus mucosus. In both cases

these organisms were found upon the first examination, and persisted throughout the entire period of examination extending over months. The capsulated streptococcus rarely killed mice, but could always be isolated from the local lesion. The Streptococcus mucosus, on the other hand, was pathogenic, and found in the blood of the infected mouse; when, however, the pneumococcus invaded the mouth, the inoculated mice died of a pure pneumococcus septicæmia (so far as could be determined from smears on serum plates), while the Streptococcus mucosus could be recovered from the local lesion or from direct smears of saliva on serum-agar plates.

One other series of examinations is of interest in connection with the persistence of certain types of organisms in the mouth. In this case the mice throughout the entire period of examination invariably died from a septicæmia caused by a Gram-negative, capsulated bacillus of the Friedländer type. The first saliva was tested December 1, 1904, the last on April 28, 1905,—a period of six months. No pneumococcus was ever isolated from this person, but in smear plates from the saliva on serum-agar a Gram-positive, capsulated coccus, displaying the characteristic growth appearance of Streptococcus mucosus, was observed.

These observations suggested to the writer the probability, mentioned before, of such organisms being permanent residents of the protected salivary ducts or the tonsillar crypts, or possibly being accounted for by their connection with slight suppurative processes of the teeth and gums.

SUMMARY AND CONCLUSIONS.

The work carried on under the auspices of the Medical Commission for the Investigation of Acute Respiratory Diseases, at the Bacteriological Laboratory of the College of Physicians and Surgeons, Columbia University, has consisted principally of a comparative study of the morphology, growth characters, fermentative activities, and agglutination reactions of pneumococci and allied organisms isolated by ourselves and the various workers under the Commission.

These organisms were from two chief sources: (*a*) from the

mouth and naso-pharynx of supposedly normal persons and persons suffering from minor inflammations of the naso-pharynx, and (*b*) from definitely pathologic sources, such as pneumonic sputum, pneumonic lungs, empyæma, the circulating blood of pneumonia patients, septicæmias, meningitis, and various minor lesions, usually due to infection with pneumococci.

One of the principal objects of the study was to make a careful comparison, in the light of the most recent knowledge of the biology of the pneumococcus and by the aid of the latest biological and technical methods, of the series of organisms from these two sources, and thus to determine definitely the true nature of pneumococcus-like organisms occurring in the mouths of normal persons.

The second and equally important object, ultimately dependent, however, upon the solution of the first, was the determination of the frequency of occurrence of typical pneumococci in the mouths of healthy individuals.

The investigations detailed in the present paper have dealt chiefly with the first problem and incidentally with the second, and lead to the following conclusions:

(*a*) That organisms, not to be distinguished by morphological characters or by any physiological peculiarities from true pneumococci derived from pathologic sources, occur with frequency in the mouths of healthy persons and those suffering from slight inflammations of the naso pharynx, and that the only permissible and legitimate conclusion is that these organisms are true pneumococci.

(*b*) That there are other organisms in normal mouths and from pathologic sources that morphologically or by staining reactions are not definitely to be distinguished from pneumococci, and can only be recognized by a careful study of their fermentative activities and agglutination reactions. These organisms are non-inulin fermenters.

(*c*) That the organism known as Streptococcus mucosus is at times found in cultivations from the mouths of apparently healthy individuals, and that, although it shows certain peculiarities distinguishing it from the typical pneumococcus, it is probably very closely related to, and a variety of, this species.

(*d*) That other organisms occur which in their fermentations are indistinguishable from pneumococci, but which either morphologically or in agglutination reactions show a variation from this type. Some of these are probably temporarily or permanently modified pneumococci or Streptococcus mucosus; others, it may be, are streptococci of types which it has not heretofore been possible to recognize and describe. Some of these organisms were isolated from pneumonic lungs at autopsy, or from some internal source such as the circulating blood, and presumably have long resided under conditions which may be considered adverse, thus bringing about a modification of their morphology or physiology. This is supported by the observation that pneumococci from such sources—perfectly typical morphologically and in fermentative activities—are apt to show a lessening of their ability to agglutinate.

In connection with the other problem, the frequency of occurrence of true pneumococci in the mouth of healthy persons, our own investigations, limited to the study of the mouths of twenty-two individuals, have given the following:

In a series of fifteen persons from each of whom one specimen of saliva only was examined, typical pneumococci were found in seven out of the fifteen specimens, i.e., 46.6 %. In the case of the remaining seven individuals of our series, repeated tests, extending over weeks and months, were made, and the pneumococcus was demonstrated upon one or more occasions in the saliva of six out of the seven, i.e., 85.7 %.

It seems, therefore, more than probable that practically every individual, at least during the winter season, when exposed to environmental conditions such as those existing in New York City, acts as the host at some time or other, and probably at repeated intervals, of organisms of the true pneumococcus type.

None of the supposedly normal individuals examined by us had had a recognized pneumococcus infection or "cold" within a recent time, nor, with two well-marked exceptions, did any symptoms of infection develop in those whose mouths were found to contain pneumococci.

These two exceptions, detailed in an earlier section, strongly

suggest the etiological relations of pneumococci to some, at least, of the "common colds."

In conclusion, it is a pleasure to thank the various investigators connected with the work of the Commission, for their courtesy in furnishing us with the cultures, upon a study of which any success our work may have attained has so largely depended.

REFERENCES.

1. Hiss. "A Contribution to the Physiological Differentiation of Pneumococcus and Streptococcus, and to Methods of Staining Capsules." *Jour. Exp. Med.*, 1905, vi, 317; abstract in *Ctbl. f. Bakt.*, 1902, xxxi, 302.

2. Hiss. "A Method of Obtaining Mass Cultures of Bacteria for Inoculation and for Agglutination Tests; with Special Reference to Pneumococci and Streptococci." *Jour. Exp. Med.*, 1905, vii, 2, p. 223.

3. Schottmüller. *Münch. med. Wochenschrift*, 1903, i, 909.

4. Rosenow. *Journ. of Infectious Diseases*, 1904, i, 308.

EXPLANATION OF THE TABLE.

The table contains a list of the organisms examined, and an outline record of the results of the morphological examinations and of the principal fermentation and agglutination tests. Where two descriptions occur under the same heading, as in the morphology and inulin fermentation columns, the first description is the one reported to us, the second is the result of our own tests.

The sera used for the agglutination tests were obtained from immunized rabbits.

The agglutination tests, recorded in the pneumococcus immune serum column, were not all made in the same serum. The letter preceding the numerals indicates the special serum in which the test was made. The agglutination of the homologous organisms in these were as follows: a—400–800; b—400–800; c—200–800; d—200–400.

The results given in the column should not, therefore, be compared with each other, except when preceded by the same letter, but with the agglutination of the homologous organism.

The other agglutination tests were made in sera from Streptococcus pyogenes and Streptococcus mucosus immune rabbits, and in the case of sera "S. 7" and "CD No. 3" from rabbits immunized against organisms which fermented inulin but were atypical morphologically and did not agglutinate to an appreciable degree in pneumococcus immune sera. The P. & S. "8" serum was from a rabbit immunized against P. & S. culture "8," which is a non-inulin-fermenting capsulated streptococcus.

When two numbers are used to record the results of an agglutination test, the first indicates the highest dilution of the serum in which a complete precipitation of the agglutinated organisms occurred, and the second the highest reading with a hand lens.

All agglutination tests were made in small test-tubes by the macroscopic method, and careful comparisons were always made with the normal rabbit-serum and salt-solution controls.

No.	Morphology.		Inulin.		Pathogenicity.	Source.	Agglutinations.						Remarks.
	Reported.	Central Lab.	Reported.	Central Lab.			Pneumococcus.	S. mucosus.	"S. 7."	"C D 3."	S. pyogenes.	P. & S. "8."	
A 1	Typ.	Typ.	+	Acid	+	Saliva, normal.	b10–200				10–50	–	
" 2	Typ.	Spher. dip.	–	–	+	Saliva, normal.	a0–20						
" 3	Spher. dip.	Spher. dip.	–	–	–	Saliva, normal.	a0–10						
" 4	Strepto.	Strepto.	–	–	+	Saliva, normal.	a–		–	–			No sugars fermented; milk slightly acid.
" 5	Spher. dip.	Spher. dip.	–	–	–	Saliva, normal.	a–		–	–			Glyco. not fermented.
" 6	Typ.	Typ.	+	+	+	Blood, pneumonia.	b50		50	–	10–50	50	
" 7	Typ.	Typ.	+	+	+	Lung, pneumonia.	b100–400						
" 8	Typ.	Typ.	+	+	+	Meninges, meningitis.	b50		10–50		0–50	0–20	
" 9	Typ.	Typ.	+	+	+	Pleura, pneumonia.	a400–800		50	–		0–50	
" 10	Typ.	Spher. dip.	?	–	+	Lung, pneumonia.	b spontaneous; all same.						
" 11	Typ.	Typ.	+	+	+	Lung, pneumonia.	b200–800						
" 12	Typ.	Typ.	+	+	+	Pus, empyæma.	b100–800						
" 13	Typ.	Typ.	–	Acid	+	Pus, empyæma.	a10–50						Involution forms marked; most fermentations slow.
" 14	Typ.;caps.?	Typ.	+	+	–	Lung, pneumonia.	a200–800						
" 15	Typ.	Typ.	+	+	+	Pus, acute otitis media.	a10						May be S. mucosus.
" 16	Typ.	Typ.	+	+	+	Lung, pneumonia.	a200–400						
" 18	Typ.	Typ.	+	+	+	Saliva, normal.	b400–800				–		
" 19	Typ.	Spher. dip.	+	–	+	Saliva, normal.	b–		–				
" 20	Typ.	Typ.;caps.?	+	+	+	Pus, empyæma.	a10–50		–	–		0–50	Involution forms.
" 21	Typ.	Gen. strepto. morph.	+	–	+	Pharynx, abscess.	a–		100–800	–		0–20	Glyco. not fermented.
" 22	Typ.	Typ.	+	+	+	Saliva, normal.	b200–800						
" 22a	Typ.	Typ.	+	+	+	Saliva, pneumonia.	a200–1600						
" 23	Typ.	Atypical.	+	+	+	Saliva, normal.	b20–400						
" 24	Typ.; no caps.	Strep.	+	–	+	Saliva, pharyngitis.	b–		–				Orange color in puncture.
" 25	Typ.	Spher. dip.; no caps.	+	+	+	Saliva, normal.	b–		20–100				
" 26	Typ.	Typ.	+	+	+	Saliva, normal.	b–800						
" 27	Typ.	Typ.	+	+	+	Saliva, normal.	b0–20						
" 28	Typ.	Typ.	+	+	+	Saliva, normal.	b200–800						
" 29	Typ.	Spher. dip.	+	+	+	Spinal fluid, meningitis.	a200–800						
" 30	Typ.	Slt. atyp.	+	+	+	Lung, pneumonia.	a10–200						
" 31	Typ.	Typ.	+	+	+	Saliva, pneumonia.	No test.	–	–	–		–	
" 32	Typ.	Typ.	+	Acid	+	Saliva, normal.	a0–50						
" 33	Typ.	Typ.	+	+	+	Saliva, normal.	a200–800						
" 34	Typ.	Typ.	+	+	+	Heart, acute endocarditis.	a100–800						

No.	Morphology.		Inulin.		Pathogenicity.	Source.	Agglutinations.						Remarks.
	Reported.	Central Lab.	Reported.	Central Lab.			Pneumococcus.	S. mucosus.	"S. 7."	"C D 3."	S. pyogenes.	P. & S. "8."	
A 35	Typ.	Typ.	+	+	+	Saliva, normal.	a400–800	0–20			100	0–20	
" 36	Spher. dip.	Spher. dip.	–	–		Blood, mastoiditis.	a10–20						No sugars fermented; milk slightly acid.
" 37	Spher. dip.	Spher. dip.; narrow caps.	–	–		Pus; hip joint.	a–						Do.
" 38	Spher. dip.	Spher. dip.	–	–	+	Peritoneum; typhoid.	a–						Orange color in punct. Glycogen not fermented.
" 39	Spher. dip.	Spher. dip.	–	–		Blood, puerperal sepsis.	a–						Do.
" 40	Coccus.	Strepto. morph; caps.	–	–		Lung, pneumonia.	a–						Do.
" 41	Spher. dip.	Spher. dip.	–	–		Pus, abscess.	a0–20						No sugars fermented; milk slightly acid.
" 42	Spher. dip.	Spher. dip.	–	–	+	Lung, consolidated.	a0–20						Do.
" 43	Spher. dip.	Mixed morph.	–	–		Lung, consolidated.	a–		–	–		–	
" 44	Spher. dip.	Spher. dip.	–	–		Lung, consolidated.	a–		–	–		–	Orange color in punct.; glyco. and lact. not fermented; milk not acid.
" 45	Spher. dip.; caps.?	Lance. dip.; caps.?	–	–	+	Saliva, slight coryza.	a–		10–50	–		–	Glyco. not fermented.
" 46	Typ.	Typ.	+	+	+	Saliva, slight coryza	a400		50–400	–		0–50	
" 47	Spher. dip.	Spher. dip.; caps.?	–	–	–	Blood, empyæma.	a–	–	–	–		–	Dextrose fermented.
" 48	Spher. dip.	Biscuit dip.	–	–	+	Saliva, pneu. 4 mos. ago.	a–	–	100–800	–		0–1/10	Glyco. not fermented.
" 49	Spher. dip.	Spher. dip.	–	–	–	Lung, pneumonia.	a–allclear.	Same.	Same.				No sugars fermented; milk slightly acid.
" 50	Typ.	Gen. strepto.	+	–	+	Saliva, normal.	a–		100–200		–	0–20	Glyco. not fermented.
" 51	Typ.	Typ.	–	+	+	Discharge, coryza.	a–		–	–			
" 53	Spher. and typ.; no caps.	Mixed morph.	–	–	+	Saliva, normal.	a–		20–800	–		–	Glyco. not fermented.
" 54	Spher. and typ.	Mixed morph.	–	–		Saliva, pharyngitis.	a0–50		20–50	20–50		0–20	Lact. and dextrose ferment.; milk coag.
" 55	Spher. and typ.	Gen. spher.; narrow caps.	–	–	–	Saliva, normal.	a–spontaneous.	Same.	Same.			Same.	Dextrose and malt. ferment.; milk slightly acid.
" 56	Typ.	Typ.	+	+	+	Saliva normal.	a200–800						

A 58a	Typ.; no caps.	Mixed morph.; narrow caps.	−	−	−	Saliva, pharyngitis; abscess, tooth.	a−		0–50	−		−	Glyco. not fermented.
" 58b	Spher. dip.	Spher. dip.	−	−	+	Saliva, normal.	No test.						Glyco. not fermented.
" 59	Spher. dip. and typ.	Spher. dip.	+	−	+	Saliva, normal.	a−						Orange color in punct.; glyco. not fermented.
" 60	Spher. dip.; caps.?	Bis. dip.; caps.?	−	−	+	Saliva, normal.	a0–10		−			−	
" 61	Spher. dip.	Lance. and bacil. forms; caps.?	−	−		Saliva, normal.	a0–10	0–20	0–20				
C D 1	Typ.	Mixed morph.; caps.	+	+	−	Lung, pneumonia.	b−	0–20		0–20		−	
" 2	Typ.	Atyp.; narrow caps.	+	+	−	Lung, pneumonia.	b−			0–50	−	−	
" 3	Typ.	Lance.; narrow caps.	+	+	−	Lung, pneumonia.	b−			20	−	−	
" 4	Typ.	Coc.	+	−	−	Lung, pneumonia.	b−		100–200	20		0–10	
" 5	Typ.	Atyp.	+	+	−	Lung, broncho-pneu.	b−		100–200	50		0–20	
" 6	Typ.	Atyp.	+	+	−	Lung, pneumonia.	b−	0–20	0–20	0–20		0	
" 7	Typ.	Atyp.	−	−	−	Lung, pneumonia.	b0–20		100–200	0–20	−	0–20	
" 8	Typ.	Atyp.	+	+	−	Lung, pneumonia.	b0–20		100–200	0–20	−	0–20	
" 9	Typ.	Spher. dip.	+	+	−	Blood, pneumonia.	b0–20		100–200	0–20	−	0–20	
" 10	Typ.	Atyp.	+	+	−	Blood, pneumonia.	b−	−	0–20	−		−	
" 11	Typ.	Atyp.		−	−	Lung, pneumonia.	a0–10				−		No sugars fermented; milk slightly acid.
" 12	Typ.	Spher. dip.		+	−	Lung, pneumonia.	a20		−	20–50			
" 13	Typ.	Spher. dip.		−		Saliva, normal?	a−	−	−	−		−	
" 14	Typ.	Spher. dip.		−	+	Lung, pneumonia.	a−	−	−	−		−	
" 15	Typ.	Spher. coc.		−	−	Lung, pneumonia.	a−		0–20	−			No sugars fermented; milk acid.
" 16	Typ.	Spher. dip.		+	−	Lung, pneumonia.	a20–50		0–10	100			
" 17	Typ.	Sl. lanceolate; no caps.		−	−	Abscess, shoulder.	a−		0–20	−			No sugars fermented; milk not acid.
" 18	Typ.	Spher. and typ.; caps.	+	+	−	Spinal fluid.	a−	−	0	0–20			
E 1	Typ.	Typ.	+	+	+	Mouth, saliva, swab, pneumonia.	b100–800	100–200					
" 2	Typ.	Typ.	+	+	+	Mouth, saliva, swab, diph.	No test.						
" 3	Typ.	Typ.	+	+	+	Mouth, saliva, swab, bronchitis.	b200–800						
" 4	Typ.	Typ.	+	+	+	Mouth, saliva, swab, grippe.	b100–800						
" 5	Typ.	Typ.	+	+	+	Mouth, saliva, swab, normal.	b200						

No.	Morphology.		Inulin.		Pathogenicity.	Source.	Agglutinations.						Remarks.
	Reported.	Central Lab.	Reported.	Central Lab.			Pneumococcus.	S. mucosus.	"S. 7."	"C D 3."	S. pyogenes.	P. & S. "8."	
E 6	Typ.	Typ.	+	+	–	Throat, swab, slight coryza.	b100–200						
" 7	Typ.	Typ.	+	+	–	Throat, saliva, swab, pneumonia.	No test.						
" 8	Typ.	Typ.	+	+	+	Throat, saliva, swab, pneumonia.	b100–800						
" 9	Typ.	Typ.	+	+	+	Throat, saliva, swab, empyæma.	No test.						
" 10	Typ.	Typ.	+	+	–	Throat, saliva, swab, pneumonia.	b100						
" 11	Typ.	Typ.	+	+	+	Throat, saliva, swab, mouth normal, chronic neph.	b200–800						
" 12	Typ.	Typ.	+	+	+	Throat, saliva, swab, normal.	b200–800						
" 13	Typ.	Small lance.; irreg. groups; no caps.	+	+	+	Throat, saliva, swab, pneumonia.	b10–20						Controls same.
" 14	Typ.	Typ.	+	+	+	Throat, saliva, swab, mouth normal, leukæmia.	b–						
" 15	Typ.	Atyp.; narrow caps.	+	+	+	Mouth, saliva, swab, pharyngitis.	b100–800						
" 16	Typ.	Typ.	+	+	+	Mouth, saliva, swab, typhoid.	b200–800						
" 17	Typ.	Typ.	+	+	+	Mouth, saliva, swab, mouth normal, cirrhosis.	No test.						
" 18	Typ.	Typ.	+	+	–	Mouth, saliva, swab, pharyngitis.	100						
" 19	Typ	Typ.	+	+	+	Mouth, saliva, swab, pharyngitis.	b200–800						
" 20	Typ.	Pn. morph.; no caps.	+	+	+	Handkerchief, pharyngitis.	–				10	200–800	
" 21	Typ.	Typ.	+	+	+	Mouth, saliva, swab, pneumonia.	b200–800						
" 22	Typ.	Atyp.	+	+	–	Lip of cup after drinking; pharyngitis.	–?	–	10?	–	–	–	
" 23	Typ	Small lance.; irreg. groups; no caps.	+	+	+	Mouth, swab, pneumonia.	–?	–		0	–	–	–

E 24	Typ.	Strept.	+	+	−	Mouth, saliva, swab, typhoid.	b50–200		20–100	50–100	0–50	50	
" 25	Typ.	Typ.	+	+	+	Mouth, saliva, swab, typhoid.	b200–800						
" 26	Typ.	Atyp.	+	+	+	Mouth, saliva, swab, lead poisoning.	b200–800						
" 27	Typ.	Typ.	+	+	+	Mouth, saliva, swab, pharyngitis.	No test.	10–100			−	−	
" 28	Typ.	Typ.	Acid.	+	+	Mouth, swab, mouth normal, grippe 4 weeks ago, rheumatism,	b100						
" 29	Typ.	Spher. dip.	+	+	+	Mouth, saliva, swab, grippe 4 weeks ago, mouth normal, rheumatism.	b0–1/10	−	10–100	−			
" 30	S. m.	S. m.	+	+	+	Mouth, saliva, swab, coryza.	b−	0–20			−	−	S. mucosus.
" 31	Spher. dip.; caps.	Strep.; narrow caps.	+	−	−	Mouth, saliva, swab, normal.	b−	−	10–20	−	−	−	
" 32	Typ.	Typ.	+	+	+	Mouth, saliva, swab, pneumonia.	a200–800	100+					
" 33	Typ.	Typ.	+	+	+	Mouth, swab, pneumonia.	a100–400						
" 34	Typ.	Typ.	+	+	+	Mouth, saliva, swab, broncho-pneumonia.	a200–800						
" 35	Typ.	Typ.	+	+	+	Mouth, saliva, swab, broncho-pneumonia.	b100–400						
" 36	Typ.	Typ.	+	+	+	Mouth, saliva, swab, pneumonia.	b100–400						
" 37	Typ.	Typ.	+	+	+	Mouth, sputum, pneumonia.	a200–800						
" 38	Typ.	Typ.	+	+	+	Mouth, saliva, swab, pneumonia.	No test.						
" 39	Typ.	Typ.	+	+	+	Mouth, saliva, swab, coryza.	b100–400						
" 40	Strept.	Atyp.	+	−	−	Mouth, saliva, swab, pharyngitis.	a20–100						Glyco. not fermented.
" 41	Typ.	Spher. dip.	+	−	−	Mouth, saliva, mouth normal, endocarditis.	b−	−		−	−	−	Said to be S. mucosus.
" 42	Spher. dip.; caps.	Strept. morph.	?	−	−	Mouth, saliva, swab, typhoid.	b−	−	0–20	−	−	−	
" 43		Spher. dip.		+		Mouth, saliva, swab, pneumonia.	b20–100	10–50	0–20	−	−	−	
" 44	Typ.	Typ.	+	+	+	Mouth, saliva, swab, tuberculosis.	b200–400						
" 44a		Typ.		+			a100–400						
" 45	Typ.	Atyp. morph.	+	+	−	Mouth, saliva, swab, mouth normal, endocarditis.	c−	−	−	−	−	−	
" 46	Typ.	Typ.	+	+	+	Mouth, saliva, swab, tuberculosis.	c200–400						

	Morphology.		Inulin.				Agglutinations.						
No.	Reported.	Central Lab.	Reported.	Central Lab.	Pathogenicity.	Source.	Pneumococcus.	S. mucosus.	"S. 7."	"C D 3."	S. pyogenes.	P. & S. "8."	Remarks.
E 47	Typ.	Small lance.; no caps.	+	−	−	Mouth, saliva, mouth normal.	c−	−	0–20	−	−	−	
" 50	Typ.	Typ. mor.; no caps.	+	+	+	Mouth, saliva, swab, pneumonia.	c100–400						
" 51	Typ.	Typ.	+	+	+	Mouth, saliva, swab, pharyngitis.	c100–400						
" 52	Typ.	Typ.	+	+	+	Mouth, saliva, swab, pharyngitis.	a800			0–20	10–50		
" 53	Typ.	Typ.	+	+	+	Mouth, saliva, swab, mouth normal, appendicitis.	a200–400 c200–400						
" 54	Typ.	Typ.	+	+	−	Mouth, saliva, swab, mouth normal, bad heart.	c100–400						
" 55	Typ.	Typ.	+	+	+	Mouth, saliva, swab, mouth normal, rheumatism.	a100–400	400–800	20–100	20–100	100		
S. M. BI.		S. m.		+			a0–10	10–20					S. mucosus.
"S. 7"		Dip. and ch.; nar. caps at times.					a0–10	10–20	200–800	−	−		
C 1	Typ.	Typ; caps.?	+	+	+	Sputum, acute tonsillitis.	b50–200				−	−	
T 3	Typ.	Typ.	+	+	+	Sputum, tuberculosis.	b50–800				−	−	
N 5	Typ.	Typ.	+	+	+	Throat, normal.	c200–800						
" 6	Typ.	Typ.	−	+	+	Throat, normal.	No test.						
" 7	Typ.	Typ.	+	+	+	Throat, normal.	c100–400						
" 9	Typ.	Typ.	+	+	+	Throat, normal.	c10–100				10–50	−	
" 10	Typ.	Typ.	+	+	+	Throat, normal.	No test.						
" 11	Typ.	Coc.	+	−	+	Throat, normal.	c−						
" 12	Typ.	Coc.	+	−	+	Throat, normal.	b0–200						
" 13	Typ.	Atyp.	+	+	+	Throat, normal.	c200–800				50–200		
" 15	Typ.	Typ.	+	+	+	Throat, normal.	c0–200				0–50	−	
" 17	Typ.	Typ.	+	+	+	Throat, normal.	c200–800	200–800					
" 23	Typ.	Typ.	+	+	+	Throat, pertussis.	c100–400						
" 36	Typ.	Typ.	+	+	+	Sputum, pneumonia.	b200–800						
" 40	Typ.	Typ.	+	+	+	Throat, normal.	a100–200		100		50–100		
" 41	Typ.	Typ.	+	+	+	Throat, normal.	b200–800						
" 43	Typ.	Spher. dip.	+	−	+	Throat, normal.	b0–10				−	−	
" 44	Typ.	Lancet.; morph.; caps. (?)	+	−	−	Throat, slight cold.	b0	−	10–20	−	−	−	
" 45	Typ.	Spher. dip.	+	−	−	Throat, slight cold.	b0–10	0–20	10–50		−		

N 46	Typ.	Spher. dip.	+	+	−	Throat, slight cold.	b100–400		100		−	−	
" 51	Typ.	Dip.; caps.	+	−	+	Throat, normal.	a−		10–50		−	−	
" 52	Typ.	Spher. dip.	+	−	−	Throat, normal.	a0–10			0–50	−	0–20	Orange growth in puncture.
" 53	Typ.	Spher. dip.	+	−	+	Throat.	a−					100–400	
" 56	Typ.	Typ.	+	+	+	Throat.	b20–50?					50–100	
" 61	Strep.	Bis. dip.	+	−	−	Throat, normal.	a0–10		−	−		20–50	
" 66	Strep.	Spher. dip.	+	−	−	Throat, normal.	a0–10		−	−			
" 74	Typ.	Typ.	+	+	+	Throat, normal.	a50–100		0–20	0–20			
" 79	Typ.; caps.?	Typ.	+	+	+	Throat, normal.	a100		100				
" 80	Strep.	Atyp.; caps. on some.	+	Acid.	+	Throat, normal.	a−		20–50				
" 81	Typ.; no caps.	Typ.	+	+		Throat, normal.	a200–800		0–20	0–20			
T 53	Typ.	Typ.	+	+	+	Throat, swab.	a10–400						
" 56	Typ.; no caps.	Gen. str. morphology.	+	−	−	Throat, swab.	a50–100		−				
" 57	Typ.	Typ.	+	+	+	Throat, swab.	a200–800						
" 59	Typ.	Typ.	+	+	−	Pus, empyæma.	a800						
" 60	Typ.	Typ.	+	+	+	Throat, swab.	a0–20						
" 68	Typ.	Spher. dip.	+	−	−	Throat, swab.	a−		100–800	0–20		0–20	Glyco. not fermented.
" 69	Typ.	Spher. dip.	+	−	+	Throat, swab.	a−		0–10	0–20		10–50	Lactose, raffinose, glycogen not fermented; no acid in milk.
" 70	Typ.	Typ.	+	+	+	Pus from pleural cavity.	a200–800						
" 72	Typ.	Spher. dip.	+	−	+	Throat, swab.	a−		−			20–50	Glyco. not fermented.
" 73	Typ.	Spher. dip.	+	−	+	Throat, swab	a−		−			20–50	Glyco. not fermented.
" 76	Typ.	Typ.	+	+	+	Throat, swab.	a10–100		50–400				
" 77	Typ.	Spher. dip.	+	−	+	Throat, swab.	a spontaneous.						Dextrose and maltose fermented.
" 81	Typ.	Typ.	+	+		Throat, swab.	a400–800		0–20	0–20		0–20	
" 82	Typ.	Typ.	+	+	+	Throat, swab.	a50–100		20–100	10			
" 83	Typ.	Typ.	+	+	−	Throat, swab.	a400–800		20	0–20			
Nor. 46	Strep.	Strep	−	−		Lung.	b20				400–1600	−	
" 49a		Typ.?	+	+		Lung.	b100–400						
" 49b		Coc.		−		Lung, broncho-pneu.	b20–100				20–100	−	Lysis of blood agar.
" 49c		Coc.	−	−		Lung.	b10–200	10–50			400–1600		Lysis of blood agar.
" 52s		Typ.	+	+		Pus, empyæma.	No test.						
" 54		Typ.		+		Lung, tuberculosis.	b100–400						
" 63i		Atyp.		−		Lung.	b−	−			−	−	Glyco. not fermented.
" 63ii		Atyp.		−		Lung.	b0–50		50–100		−	−	Do.
" 63iii		Atyp.; caps.		−		Lung.	b−				−	−	Do.
" 71	S. m.	S. m.	+	+	+	Lung, emphysema.	b0–10		0–10		10–100	10–20	S. mucosus.
" 102	Dip.	Lancet.; no caps.	+	+		Pneumonia.	b−		0–20			0–20	
" 107	Typ.	Typ.	+	+	+	Exudate, pneumonia.	b100–400		0–20				
" 199	Dip.	Lancet.; no caps.	+	+		Mouth, pneumonia.	b50–400		20–50				Controls slight reaction.

No.	Morphology. Reported.	Morphology. Central Lab.	Inulin. Reported.	Inulin. Central Lab.	Pathogenicity.	Source.	Agglutinations. Pneumococcus.	S. mucosus.	"S. 7."	"C D 3."	S. pyogenes.	P.& S. "8."	Remarks.
Z 1		Typ.		+		Blood, pneumonia.	a50–200		0–20	0–10			
" 2		Typ.		+		Sputum, pneumonia.	a0–10		—	—			
" 3		Typ.		+		Blood, pneumonia	a100–400						
" 4		Bis. dip.		—		Sore throat.	a0–10		0–20	0–10			Probably M. tetragenus.
" 5		Strept.		—		Throat, normal.	a small clumps in all.		Same as Pn.	Same as Pn.			
" 7		Typ.		+		Lung, pneumonia.	a100–400						
" 8		Typ.		+		Blood, pneumonia.	a100–400						
" 9		Typ.		+		Blood, pneumonia.	a spontaneous.	10–100	Clumps in all.	Same.			Many involution forms.
" 10		Spher. and typ.		+		Sputum, pneumonia.	a400–800		20				
P. & S. 1		Typ.		+		Sputum, pneumonia.	b200–800	200–800	50–400	—	0–100	—	
" 2		Typ.		+		Sputum, pneumonia.	No test.						
" 3		Typ.		+		Sputum, pneumonia.	b400–800	200–800			0–100	—	
" 4		Typ.		+		Eye, keratohypopyon.	No test.						
" 5		Typ.		+		Sputum, pneumonia.	No test.						
" 6		Typ.		+		Sputum, pneumonia.	No test.						
" 7		S. m.		+		Saliva, normal.	a—	10–200	—	—	0–20	—	S. mucosus.
" 8		Gen. strept.; caps.		+		Saliva, normal.	a—	—	50–100	—	0–20	50–100	
" 9		Typ.		+		Saliva, normal.	b400–800						
" 10		Typ.		Acid.		Saliva, slt. "cold."	No test.						
" 13		Typ.		+		Saliva, normal.	d200–400						
" 15		Typ.		+		Saliva, slight catarrh.	?				—		
" 16		Typ.		+		Saliva, normal.	d100–200						
" 17		Spher. dip.		—		Saliva, normal.	d—						
" 21		Typ.		+		Saliva, normal.	No test.						
" 22		S. m.		+		Saliva, normal.	bo–10	10–50	—		—		S. m. from same mouth as No. 7.
" 23		Typ.		+		Saliva, normal.	b100–800	100–200	50–400	0–20			
" 24		Typ.		+		Saliva, normal.	b400–800				100		
" 26		Coc.		—		Saliva, normal.	No test.						
" 27		Spher. dip.		—		Saliva, normal.	b20–50						
" 30		S. m.		—		Saliva, normal.	bo–10	10–20			0–20		S. m. from same mouth as No. 7.
" 31		Spher. coccus.		—		Saliva, normal.	b—				—		
" 32		Typ.		Acid.		Pleural fluid, pneu.	b20–200				20–100		
" 33		Typ.		+		Saliva, normal.	b800						
" 34		Atyp.		—		Saliva, normal.	b—	—	—	—	—	—	

P. & S. 35		Typ.			+		Saliva, normal.	b200–800						
" 36		Typ.			+		Sputum, ± tuberculosis	b400–800				50–100		
" 39		Typ.			+	+	Saliva, normal.	b200–800						
" 40		Typ.			+		Saliva, normal.	b200–400						
" 41		Typ.			+		Saliva, cold 1 mo. ago.	b50–800						
" 42		Friedl.			–		Saliva, normal.	No test.						Friedländer bacillus.
" 43		Typ.			+		Saliva, normal.	b400–800						
" 44		Gen. strept.; caps.			–		Saliva, pharyngitis.	b0–50				–	100–800	Same mouth as No. 8.
" 45		Typ.			+		Saliva, coryza.	b400–800	100–200					
" 47		Typ.			+		Sputum, "catarrh."	b50–400				20–100		
" 48		Dip. bac.; caps.			–		Sputum, cold.	No test.						
" 49		Spher. dip.			–		Saliva, normal.	a–	–	0–10	0–10			
" 50		Typ.; no caps.			+		Saliva, pharyngitis.	a0–10?						
" 51		Typ.			+		Nasal secretion; pharyngitis.	a200–400						
" 53		Typ.			+		Saliva, pharyngitis.	a200–400						
" 53b		Spher. dip.			–		Normal.	a–		–	–		20–50	
" 54		Gen. strept.; caps.			–		Saliva, cold, just recovered.	a–		–	–		10–20	Same mouth as No. 8. and No. 44.
" 54a		Gen. strept.; caps.			–		Saliva, cold, just recovered.	a–					20	Same as above.
" 55		Typ.			+	+	Saliva, normal.	a100–400						
" 55a		S. m.			+	–	Saliva, normal.	a0–10	10–50				20	S. mucosus from same mouth as No 7.
"Floyd."		Typ.			–			b. all clear.					All clear.	
"Jessup Sep."		Typ.			+			a20–100						
"Dit. Men."		Typ.			+			a100–400						
"W. 1."		Typ.			+			b200–800						
"W. 2."	Typ.	Typ.		+	+		Spinal fluid, meningitis.	a200–800						
"S. pyog. No. 1."		Strept. morph.			–			200–800				800–6400	–	

THE VIABILITY OF THE PNEUMOCOCCUS AFTER DRYING: A STUDY OF ONE OF THE FACTORS IN PNEUMONIC INFECTION.

By FRANCIS CARTER WOOD, M.D., of New York,

Adjunct Professor of Clinical Pathology, College of Physicians and Surgeons, Columbia University; Pathologist to St. Luke's Hospital.

(*A Study from the Department of Pathology, Columbia University, under a Grant from the Commission for the Investigation of Acute Respiratory Diseases, of the Department of Health of the City of New York.*)

The exact way in which the pneumococcus reaches the lungs of persons suffering from pneumonia due to that organism is not yet thoroughly understood. A number of possibilities have been considered which may be indicated as follows:

(1) The first is that the pneumococcus is frequently present in the saliva, and that when the resistance of a person carrying these organisms is reduced, for example by exposure or overwork, an infection of the lungs takes place either by extension along the tracheal mucosa or by the direct aspiration into the lung of particles of the salivary secretion carrying the germs with them.[1]

(2) A second possibility which may be considered is that the pneumococcus is transferred from the oral or pharyngeal mucosa to the lungs by the lymphatics or through the blood.

(3) Another possibility is that the pneumococcus, which is capable of living in masses of dry sputum for some time, is distributed in the form of dust derived from the dried sputum particles and that these particles are inhaled, thus giving rise to a pulmonary infection.

(4) A fourth suggestion is that the pneumococcus is carried

[1] For phases of this problem which cannot be considered here, see Wadsworth, *American Jour. of the Med. Sciences*, 1904, cxxvii, 851. Other papers on the subject are: Nenninger, *Zeit. f. Hyg.*, 1901, xxxviii, 94; Klipstein, *Zeit. f. klin. Med.*, 1898, xxxiv, 191; Dürck, *Deutsches Arch. f. klin. Med.*, 1897, lviii, 368; Wandel, *ibid.*, 1903, lxxviii, 1.

directly from person to person either by the transfer of the normal nasal or salivary fluids, which may contain pneumococci, by coughing or sneezing, or by the spraying of fine particles derived from the sputum of those suffering from pneumonia or other acute inflammations of the air passages, by the same mechanical processes, and that the spray particles thus formed carry virulent organisms to the lungs.

The present study is devoted to a consideration of the possibilities of the aërial transmission of the pneumococcus either in the form of sprayed particles or as dust derived from dried sputum, the modes of infection from the saliva as given in the first and second paragraphs not coming within the scope of this investigation.

While a good deal of work has been done by Cornet, Flügge, and others in determining the viability of the tubercle bacillus and other organisms in fine spray and also after drying and subjection to various physical agents, but little attention has been directed to the pneumococcus except when dried in relatively large masses of sputum. Most observers have considered the pneumococcus as an organism incapable of living for any considerable time when suspended in the form of fine spray.

As the results of recent studies [2] on the biology of the pneumococcus have rendered the identification of that organism relatively easy, and as some of the earlier studies on the viability were carried out with bacteria which may or may not have been the pneumococcus, it seemed to the writer that a revision and extension of some of the older investigations might be of value in deciding some points in the mode of transmission of this organism which, though important, have not yet been cleared up.

HISTORICAL RÉSUMÉ.

Before proceeding to the description of the methods and results of personal experiments, it may be well to give a short résumé of the work done by other observers on the general question of transmissibility of the pneumococcus from infectious material to human beings.

[2] Hiss, *Jour. of Exper. Med.*, 1905 vi, 317.

Viability of Pneumococci in Dried Sputum.—The earlier experiments to determine the dangers of air infection by the pneumococcus were conducted with the idea of fixing the length of time during which the organism would remain virulent for rabbits or mice after drying sputum in bulk, the powdering and diffusion of the powder by air currents being thought to be the means of transmission.

It was known that the pneumococcus died very rapidly in many of the ordinary culture media. In fact it was pointed out by Kruse and Pansini [3] that some varieties of bouillon made from meat infusion were highly bactericidal to the pneumococcus. The same observers found that in body fluids, however, for example in sealed tubes containing pleuritic exudate, the pneumococcus may remain alive for more than a year, if kept in a cool, dark place. They showed that in moist sputum preserved at 15° C. the life of the organism is very short, usually but three or four days. In sputum kept within a few degrees of 0° C., however, the life of the organism is much longer, and while the fluid loses in a few days much of its virulence for mice, yet living pneumococci can be demonstrated for at least six weeks under these conditions.

Drying of the sputum in the air at incubator temperatures killed the organisms quickly, though Guarnieri [4] found that rapid drying in a desiccator at 37° C. preserved the virulence for rabbits for four months.

Patella [5] noted that rapid drying over sulphuric acid at 16° C. or 38° C. killed the organisms promptly, while slow drying at low temperatures enabled them to live for some time. As it has been shown by Kirstein [6] that sulphuric acid probably gives off a small quantity of sulphur trioxide, which would act destructively upon any organisms with which it might come into contact, the rapid death frequently observed when the pneumococcus is desiccated over this medium may be due to the bactericidal

[3] *Zeit. f. Hyg.*, 1892, xi, 279.
[4] *Atti della R. Accad. med. di Roma*, 1888, iv, 97.
[5] *Ibid.*, 447.
[6] *Zeit. f. Hyg.*, 1902, xxxix, 166.

action of the acid. Drying over calcium chloride and phosphoric anhydride does not destroy bacteria so quickly as drying over sulphuric acid.

Foà and Bordoni-Uffreduzzi [7] dried rabbit's blood containing pneumococci on watch glasses and found that the micro-organisms were alive and virulent after forty-five days. Agar tubes inoculated from organs and kept for sixty days showed an abundant growth when placed in the incubator—an evidence that the organisms may remain alive under suitable conditions. At the time at which this work was done, however, the difference between the pneumococcus and the meningococcus had not been thoroughly defined, and the writers termed the organism with which they worked a meningococcus because it was obtained from a case of cerebrospinal meningitis. From their description of its biological features, however, the organism seems to have been Diplococcus pneumoniæ. It was positive to Gram, showed a capsule, and killed rabbits promptly. Apparently without recognizing their nature, the writers figure pneumococci inclosed in the phagocytic cells of the pneumonic exudate of the rabbit.

Five years later Bordoni-Uffreduzzi [8] reported the results obtained by contaminating pieces of linen with pneumonic sputum. The cloth was allowed to dry at room temperatures. In one specimen exposed to diffuse light the bacteria remained alive for nineteen days, as determined by injection into rabbits of scrapings from the cloth; in another, fifty-five days. The sputum dried in sunlight was virulent after twelve hours. The results differ somewhat from those of Patella, possibly depending upon the technique, Patella using silk threads soaked in the blood of an animal dying of a pneumococcus septicæmia or threads soaked in broth cultures of the pneumococcus.

The results of Cassedebat [9] differ considerably from those of Bordoni-Uffreduzzi. The experiments were conducted as follows: Sputum was tested for its virulence and found to kill rabbits. Specimens of this pneumonic sputum were then dried

[7] *Zeit. f. Hyg.*, 1888, iv, 67.
[8] *Arch. p. l. sc. med.*, 1891, xv, 341.
[9] *Revue d'Hygiène*, 1895, xvii, 1066.

on cloth in the air but protected from the direct rays of the sun. Fragments of the cloth were soaked in water and the fluid injected into a rabbit. The results showed that the dried sputum killed rabbits at periods varying from five to twenty-six days, and that fresh sputum from the eighth and ninth days of the disease would not kill rabbits. Apparently the writer relied on the gross post-mortem findings for the identification of the pneumococcus. There is no mention of morphological studies of the blood to identify capsulated organisms or attempts to cultivate the pneumococci. The results have, therefore, but slight value.

Ottolenghi[10] repeated the studies of Bordoni-Uffreduzzi with the following results. The experiments were carried out with three specimens of pneumonic sputum from the fourth or fifth day of the disease. The sputum was spread on linen cloth and allowed to dry in diffuse light at a temperature of 15° C. to 20° C. In explaining the results which he obtained, the author calls attention to the fact that the inoculation of the material into a rabbit is not sufficient to determine whether the pneumococcus is dead or not. The death of the rabbit merely determines the presence of organisms virulent for that animal, but non-virulent forms may be present. He therefore made cultures from the sputum at the same time that he carried out the animal inoculations. The first specimen tested lost its virulence for animals thirty-six days after the preparation was made, whereas pneumococci could be obtained culturally for sixty days after drying. In the second specimen, both methods showed pneumococci at the end of seventy days, and from the third specimen pneumococci were isolated on the eighty-third day. On the basis of these experiments, the author considered that Diplococcus lanceolatus can retain its virulence in dried sputum for at least twenty days, and that it remains alive for a considerable time after the virulence has disappeared. The virulence persisted longest in a thin, frothy sputum.

In some experiments recently reported by Heim,[11] the viability

[10] *Cent. f. Bakt.*, 1899, xxv, Abt i, 120.
[11] *Zeit. f. Hyg.*, 1905, *l*, 123.

of the pneumococcus after drying was much greater than has usually been assumed. Silk threads were dipped into the heart's blood of cats, rabbits, and mice, which had been killed by injections of pneumococci. The threads were dried in a desiccator over calcium chloride, and were then removed at various periods, placed in bouillon or agar, and the resulting culture inoculated into mice. The organisms were virulent in some cases after 487 days. Great variations, however, were observed. Some of the cultures from the threads no longer gave rise to septicæmia after sixty-six days. One case was not virulent after nine days. In empyema pus the pneumococcus remained virulent for 377 days. Another specimen of empyema pus contained organisms which Heim states lie between the pneumococcus and the streptococcus groups. These were virulent at the end of 149 days and contained viable organisms at the end of 383 days. The conditions of these experiments are, however, highly artificial and cannot be considered as applying very definitely to the question of aërial infections. The alternate drying and moistening of the organisms due to the varying amounts of moisture in the air of rooms is very important as determining the rapid death of the pneumococcus, while protection from such changes by sealing the substances carrying the bacteria in vessels containing calcium chloride tends to prolong the life of the parasite.

Mode of Distribution of Dried Sputum Particles.—It thus having been conclusively shown that the pneumococcus can remain alive for a considerable length of time in dried sputum, it is necessary to demonstrate that this dried sputum, which under ordinary conditions is firmly adherent to the substance on which it is dried, can in some way be reduced to a powder and thus inhaled. Such conditions can only be realized when the sputum is dried in handkerchiefs, bedding, or clothing, and the contaminated material handled, or when the sputum is deposited upon the floor and pulverized by persons walking over the infected area, or distributed in the air by dry sweeping of the floor, or brushing of infected clothes, etc. This mode of distribution of infectious material has been studied chiefly in connection with the tubercle bacillus, because of the ease of identifying that organism in the

infectious dust, and the difficulty attendant upon the recognition of the pneumococcus under the same conditions. The results obtained, however, can be legitimately transferred to the pneumococcus, leaving out of the question for the moment the viability of the latter organism after drying. Much of our knowledge on this subject we owe to the studies of Cornet and of Flügge and his pupils.

Some of the results which have been obtained are as follows: Cornet,[12] who considers this dust inhalation the most important means of infection in tuberculosis, has demonstrated the infectious nature of the dust of rooms in which persons suffering from tuberculosis had lived, and showed that the risk of infection depended very largely upon the expectoration of the sputum on handkerchiefs, bedding, carpets, or clothing, and the subsequent drying of the fluid. He considered that there was practically no danger of direct infection in tuberculosis by particles of sputum expelled by coughing, but that the sputum expectorated in large masses and dried on the bedding or floor was the chief source of the disease. His results have been disputed by Fränkel and also by Flügge and his pupils, who have shown that it is difficult to pulverize the sputum to a sufficient degree to produce a powder fine enough to be carried by air currents of moderate velocity or to remain long in suspension.

Sticher,[13] in order to test this, pulverized dried tuberculous sputum and found that while the particles could be carried by a current of air with a velocity of 1 cm. per second to a height of one meter, yet the number of bacteria which even the very fine dust particles carried was small, and hence infection was not likely to result from the dissemination of such dust. The air currents in rooms without special ventilation rarely exceed one centimeter per second and do not transport for any length of time coarse dust such as is produced by powdering sputum.

Beninde[14] repeated the experiments of Sticher and found that

[12] *Die Tuberculose, Nothnagel's Spec. Path. u. Therap.*, Bd. xiv, p. 209. Vienna, 1899.

[13] *Zeit. f. Hyg.*, 1899, xxx, 163.

[14] *Ibid.*, 193.

using handkerchiefs contaminated with tuberculous sputum it was impossible while the latter was still damp to remove any bacilli from the surface of the cloth by a stream of air with a velocity of 10 cm. per second, this being the upper limit of air currents in well ventilated rooms. Tubercle bacilli, however, could be removed by using an air stream of 1 cm. per second, but only after the handkerchief had been carried about for two days and was thoroughly dry.

Further investigations in this line were made by Neisser,[15] who used pneumonic sputum and studied the conditions obtained by mixing the fluid with dust and drying. This combination was then finely pulverized and carried from one chamber to another by an air current with a speed of from 2.8 mm. per second to 23 cm. per second. As a control, some of the sputum used was injected into mice and shown to be virulent. As soon as pneumonic sputum and the dust mixture dried, it would no longer kill mice, therefore the danger of dust infection by inhaling pulverized and dried sputum seemed exceedingly remote.

It should be repeated, however, that while it is not difficult to obtain dry and finely pulverized pneumonic sputum under experimental conditions, yet practically the drying of the mass is rarely complete enough to permit thorough powdering, and the particles which are removed mechanically from the sputum, after this fluid has dried on cloth, wood, or metal, are of such dimensions that they cannot be carried for any considerable distance by the air currents ordinarily found in well ventilated rooms, or, if so carried, remain in suspension for a very short time. The contaminated particles are not likely, therefore, to be inhaled even by persons in close contact with the patient, and are still more unlikely to lead to an infection of persons in other rooms or at a distance. Only in structures subject to strong draughts, such as factories or railroad carriages, are air currents likely to be strong enough to render these coarse particles dangerous. The possibilities of infection are reduced to a minimum when the dust particles are blown about in the open air. The dilution is so great and the death of the organisms

[15] *Zeit. f. Hyg.*, 1898, xxvii, 175.

contained in the dust is so rapid that infection cannot be assumed as likely to occur.

Germano [16] studied the effect of drying the pneumococcus with dust by a different technique. The results which he obtained with cultures of the pneumococcus showed that when mixed with sterile dust and dried, the organisms died within two days, unless the drying took place at a temperature below 0° C., when the organisms remained virulent for eight days. Mixed with sand and vegetable soil, the organisms died in two days; mixed with volcanic ash (Tuffboden), and kept moist, the organisms remained alive for six days.

Another group of experiments with another organism, presumably pneumococcus, showed a very considerable variation from the above. The culture mixed with brick dust remained infectious for forty days, either kept moist, dried in the air, or dried over sulphuric acid. Mixed with sand this same organism was infectious for sixty days when kept moist or dried at room temperature, and for fifty days when dried over sulphuric acid. This organism was obtained during an epidemic of pneumonia which occurred in a small village, a number of cases developing about the same time. Germano thinks that possibly the epidemic was due to the long life of this organism in the air.

The results of experiments by the same writer with pneumonic sputum confirmed the facts which have been observed as to the long life of the bacteria when dried in coarse particles. For example, pneumonic sputum mixed with room dust kept moist was virulent for twelve days, kept dry, for twenty days, dried over sulphuric acid, for sixteen days, dried at a low temperature, for eight days. The last results are thus somewhat different from those obtained by Patella and Neisser. Sputum mixed with earth (Humusboden) was virulent for twelve days when kept moist, for one hundred and forty days when dried in the air, and for one hundred days when dried over sulphuric acid. With a low temperature the virulence was retained for only sixteen days.

The writer considers that slight variations in the type of the

[16] *Zeit f. Hyg.*, 1897, xxv, 439; *ibid.*, 1897, xxvi, 66 and 273.

diplococcus may contribute very largely to the length of time during which the organisms can resist drying. It was certainly proven that they remained virulent longer when dried than when kept moist. At low temperatures the short life of the organisms seemed to be conditioned by the fact that the sputum dried slowly at a point near 0° C. so that the bacteria were really kept moist. The rapidity of the drying process at room temperature had no influence upon the life of the diplococcus. Germano concludes, finally, that air infection of human beings by the organism is possible with the pneumococcus, but the chances are relatively small.

It will be seen from the preceding résumé that the views expressed by Cornet on the possibility of dust infection in tuberculosis can hardly be considered as of great import in pneumonia. The sputum of pneumonic patients is often exceedingly viscid and thick; it is not usually produced in the same abundance as in pulmonary tuberculosis; and the coughing of the patients is rarely so prolonged or strongly expulsive. Coarse particles are therefore less likely to be distributed in the neighborhood of such a patient. When such particles are expelled, the results of Sticher and Beninde show that the air currents which are ordinarily present in well ventilated houses are insufficient to remove bacteria from the moist or dried sputum. The risk of infection must be largely confined to those who handle the bedding, etc., of pneumonic patients.[17] The experiments of Neisser and Germano are not wholly consistent, but tend to show that the pneumococcus dies early when dried in finely divided sputum. It is possible that in some of Germano's experiments the organism was not the one which we are accustomed to consider as the pneumococcus.

Conditions under which the Pneumococcus may be Transmitted in Sprayed Particles.—We are largely indebted to Flügge and his co-workers for the experimental investigation of the theory that the transfer of pathogenic bacteria from one person to another is

[17] See in this connection a report by Edson and Ghriskey on "A Hospital Epidemic of Pneumococcus Infections," *Trans. of the Philadelphia Coll. of Phys.*, 1904, xxvi, 6.

possible by the aërial transmission of fine spray particles. They considered that the spraying of fine particles of sputum of saliva by talking, coughing, sneezing, or spitting, might carry infectious material from one person to another if the specific organism remains alive in such spray for a sufficient time to permit the floating particles to be carried from the patient to other persons in the vicinity by the air currents produced for purposes of ventilation.

The studies of this means of transmission of the pneumococcus are not numerous, and we have obtained a large part of our knowledge concerning such transmission by investigating the conditions of infection which obtain in connection with pulmonary tuberculosis. Here again most of the factors are the same with both the pneumococcus and the tubercle bacillus, and we can without impropriety transpose the results obtained by the study of one organism to another.

While it has been repeatedly shown that the air expired from the lungs during quiet breathing contains no bacteria, yet in disease a small number of bacteria may be given off. It has been shown by Koelzer[18] that tuberculous persons by a sort of internal spraying give off tubercle bacilli even during quiet breathing, and that the fine drops containing tubercle bacilli are produced by the pulverizing of the thick mucus in the bronchi during the passage of the expiratory current—the same physical condition which gives rise to the râles heard on auscultation of the chest. Laryngeal tuberculosis increases the liability to the contamination of the expired air. Koelzer obtained positive results in one case out of fifteen persons examined, care being taken to see that no coughing took place while the Petri dishes were exposed to the expiratory current. A similar observation is recorded by Schäffer,[19] who was able to demonstrate lepra bacilli in the expired air of persons suffering from lesions of the nose and throat due to that organism.

The results of many observations on the bacterial content of the expired air have shown, however, that this source of infection

[18] *Zeit. f. Hyg.*, 1903, xliv, 217.

[19] *Arch. f. Derm. u. Syphilis*, 1898, xliii and xliv, 159.

may be practically neglected. On the other hand, coughing and especially sneezing, as previously mentioned, cause an abundant spraying of fine fluid particles which may contain bacteria.

Koeniger [20] has studied with great care the conditions under which this spraying takes place. Large numbers of particles are produced when the expiratory stream is interrupted and then suddenly begun. The drops are formed in the portion of the respiratory tract where the stoppage of the air current takes place. Particles produced in the larynx are often stopped by the lips so that the method which the person uses in coughing causes great variations in the number of particles expired in the air. If the mouth be kept nearly closed, the laryngeal particles do not escape in large numbers. If, however, the mouth is tightly closed, drops may be produced from the passage of the air currents over the lips, as the latter are pushed open by the cough. Particles are also sprayed out in clearing the throat. Loud speaking gives more spraying than quiet conversation, and the method of articulation exerts considerable influence. The letters k, p, f, and t cause more spraying than vowels or other consonants. It is probable that more germs are sprayed from a thin, watery sputum than from a thick, mucous variety, but the intensity of the coughing impulse is much more important than the consistence of the sputum. More particles are expelled when the cough is short and sharp. Koeniger's studies on spraying were made by infecting the mouth of the experimenter with B. prodigiosus and other saprophytic organisms and then exposing large numbers of plates while talking, coughing, or sneezing.

Dimensions of the Sprayed Particles.—The size of the particles produced by coughing varies greatly. Heymann [21] studied the size and number of the drops by catching the spray produced by coughing on glass slides and measuring the size of the drops so produced. The finer particles had a diameter when flattened on glass of from thirty to forty micra. Sneezing, according to my own observations, may give rise to a very fine spray, the particles not measuring over ten to twenty micra. The same is true of the

[20] *Zeit. f. Hyg.*, 1900, xxxiv, 119.

[21] *Ibid.*, 1899, xxx, 139.

drops produced in a spray apparatus using either a hand bulb or air at high pressure. Many of the particles are very small and contain no bacteria. Those above twenty micra usually contain one or more organisms if the sputum is rich in bacteria. Even the smaller particles, however, though they may not contain bacteria, when collected and examined are found to have a nucleus formed either by mucus or by salt crystals. The evaporation from these small particles when sprayed into the air is exceedingly rapid, because of their small size.[22]

Spatial Distribution of the Sprayed Particles.—Flügge in a series of papers[23] has shown that a person with a cough sprays fine particles into the surrounding air, the radius of the zone of such spraying being usually one meter, rarely two meters. Within this area therefore the air may contain floating particles carrying pathogenic bacteria. Flügge has shown that fine dust particles laden with bacteria may be carried horizontally by a stream of 0.2 mm. per second, one five-hundredth of the speed of a barely perceptible draught. Upward translation of these motes requires a slightly greater wind velocity, about 0.3 to 0.4 mm. per second. Stronger currents of air may carry them to great distances. Hutchinson[24] was able to demonstrate the transportation of particles containing B. prodigiosus for a distance of 600 meters. The drops produced by coughing, sneezing, etc., are usually larger and heavier than those just mentioned, and Heymann[25] has shown that a large proportion of them settle out of the air of an ordinarily ventilated room within an hour. Particles of this size are not transported laterally to any very great extent. In fact, it is exceedingly difficult to demonstrate tubercle bacilli in the air of wards containing tuberculous patients.

As the sprayed particles settle they adhere to the furniture, walls, bedding, and carpets, and dry. It is then impossible to remove them by any stream of air within the limits of ordinary

[22] See on this point Thomson, Conduction of Electricity through Gases, Cambridge, 1903, p. 135.

[23] *Zeit. f. Hyg.*, 1897, xxv, 179; *ibid.*, 1899, xxx, 107; *ibid.*, 1901, xxxviii, 1.

[24] *Ibid.*, 1901, xxxvi, 223.

[25] *Ibid.*, xxxviii, 21.

ventilation currents. It is possible to remove these particles, however, by dry brushing, sweeping, or dusting, and the powder so formed may float for a long time in the room or be transported to adjacent ones.

Bacterial Content of Sprayed Particles.—Direct evidence of the bacterial content of the sprayed sputum has been obtained by B. Fränkel, who examined the contents of two hundred and nineteen face masks each of which had been worn for twenty-four hours by persons whose sputum had contained tubercle bacilli. In twenty-six of these masks tubercle bacilli could be demonstrated. Fränkel assumes that in thirty-two days 2600 tubercle bacilli had been caught in masks which would otherwise have escaped into the air. In a considerable number of cases of tuberculosis, however, Fränkel pointed out that the masks remained uninfected so that only a small number of patients could be shown to cough out drops of sputum or saliva containing tubercle bacilli.

The presence of virulent organisms in sprayed sputum has been verified by other observers.[26] The most detailed study is perhaps that of Heymann,[27] who examined with especial care the conditions attending the spraying of tuberculous sputum by patients under, so to speak, natural conditions—that is, the coughing was not forced, the patients simply being confined to a room while the tests were carried out. The particles sprayed out by the patients were collected and found to contain abundant tubercle bacilli.

An interesting example of the large numbers of bacteria which may be expelled is that reported by Schäffer [28] where one leprous patient at a single sneeze gave off 25,000 bacilli, and another patient, 110,000. Patients with severe lesions of the tuberculous type gave off from 10,000 to 185,000 lepra bacilli in ten minutes' talking. The sprayed bacteria were caught on slides placed close to the patients' mouths and only a very few could be demonstrated at a distance of one and one-half meters.

[26] v. Hübener, *Zeit. f. Hyg.*, 1898, xxviii, 348; v. Weismayer, *Wiener klin. Woch.*, 1896, p. 1039; Bing, *Zent. f. innere Medizin*, 1905, p. 54; Mendes de Leon, *Arch. f. klin. Chir.*, 1904, lxxii.

[27] *Zeit. f. Hyg.*, 1901, xxxviii, 21.

[28] *Arch. f. Derm. u. Syphilis*, 1898, xliii and xliv, 159.

Hamilton [29] finds that streptococci are expelled from the mouth by coughing or even by breathing by persons with streptococcus infection of the upper air passages.

Life of the Bacteria in the Spray Particles.—In order to study the length of life of bacteria in sprays, Laschtschenko [30] atomized diluted pneumonic sputum (ten parts sputum and from one to two parts water) in a closed vessel and caused the particles to be carried upwards for one meter by a current of air of from 6 to 10 mm. per second.

The spraying was continued under low pressure for one and one-half hours. The particles were collected and the fluid injected into mice, with three positive and two negative results. Spraying *undiluted* sputum with air speeds of 10 to 12 mm., he obtained one positive and six negative results, the infectious nature of the sputum being previously determined by injecting mice. Using the same apparatus and conditions with phthisical sputum, a positive result was obtained in all cases with air speeds of from 6 to 14 mm. per second, the sputum being diluted and undiluted. The spray was produced by a very low air-pressure stream. The results show that the pneumococcus and the tubercle bacillus can live for a longer or shorter time in sprayed sputum. The writer gives no explanation of the fact that many more positive results were obtained with tuberculous sputum than with the pneumococcus, but it is evident from my own experiments, to be given later, that the drying which the pneumococci underwent while carried up in the air current was sufficient to kill many of the organisms.

A phenomenon noted by Koeniger [31] is of interest in this connection. He observed that after spraying large quantities of cultures of B. prodigiosus over the floor and furniture of a room it was impossible to obtain colonies of this organism on exposed plates even when large amounts of dust were produced by energetic brushing. Growths were obtained from many other organisms but not from the prodigiosus. This fact was not fully understood by Koeniger, who states that the results of his

[29] *Jour. of the American Med. Assoc.*, 1905, p. 1108.
[30] *Zeit. f. Hyg.*, 1899, xxx, 133. [31] *Ibid.*, 1900, xxxiv, 119

experiments show that the bacteria must be moist to produce a growth. The true explanation was given shortly afterward by Kirstein, who showed that the reason for the negative results was that B. prodigiosus when sprayed in fine particles dried rapidly and was promptly killed, especially when exposed to diffuse daylight. In one set of experiments by the latter, B. prodigiosus was sprayed in two rooms and the falling germs caught on glass plates. In the dark room the bacteria remained alive for fifteen days, in the well-lighted room for only three days.

Similar results were obtained by Kirstein[32] for pathogenic organisms such as the typhoid bacillus, which remained alive for only a few hours, the tubercle bacillus, which was alive for from four to eight days in diffuse light and as long as forty days in the dark.

Staphylococcus pyogenes aureus and streptococcus remained alive for from ten to sixteen days; diphtheria bacilli, less than twenty-one hours; anthrax bacilli, nearly ten weeks. The pneumococcus was not investigated, as the author assumed from the results of previous studies by Neisser and others that prompt death of the pneumococcus occurred after drying.

In a more recent paper, Kirstein[33] finds that the tubercle bacillus lives for from eight to fourteen days when sputum is sprayed on fine dust, from four to seven days when tuberculous sputum is finely powdered, five days when deposited on fine cloth fibers, and finally that the bacillus lives but three days on fine street dust although it was alive for eight days on coarse dust of the same variety. In all of these tests the bacteria were exposed to diffuse daylight.

All observers are agreed then that the life of the bacteria when sprayed and dried may be safely assumed to be much shorter than when they are dried in masses. Diffuse light and especially sunlight rapidly destroy the organisms, while preservation in a dark, cool place tends to prolong their existence.

[32] *Zeit. f. Hyg.*, 1900, xxxv, 123; *ibid.*, 1902, xxxix. See also Ficker, *Zeit. f. Hyg.*, 1898, xxix, 1.

[33] *Zeit. f. Hyg.*, 1905, l, 186.

Summary.—As will be seen from the survey of the bibliography of the subject just given, the conditions of the viability of the pneumococcus have been fairly well established when either sputum or other fluids containing the organism are dried in bulk and exposed to diffuse daylight or the direct rays of the sun. There are minor inconsistencies in the results dependent upon the method used, the sensitiveness of the animal employed to determine the presence of living pneumococci, and possibly also upon slight variations in resistance of the various strains. The identification of the organism was, however, so far as is reported in many of the studies, entirely dependent upon either the morphology of the bacteria isolated or even upon the death of the animal without any microscopical verification of the presence of a septicæmia. As it has been shown that there are other capsulated organisms which are fatal to mice if given in sufficiently large amounts, and as these animals and also rabbits frequently die after the injection of sputum without the presence of pneumococci being determinable either morphologically or by culture, it seemed to the writer that a few experiments might properly be devoted to a repetition of the studies of the earlier Italian and German workers whose papers have already been considered. The experiments of Germano in mixing cultures of the pneumococcus or sputum with sterile dust were not repeated for they are very complete as they stand and are not especially pertinent to the question in hand.

The main portion of the writer's studies were therefore devoted to the investigation of the question of fine sprayed particles containing pneumococci and the length of life of the organisms in this spray. This ground has not been fully covered by previous workers, and as its great importance in the transmissibility of the tubercle bacillus has been shown, it seemed proper to extend our knowledge to the pneumococcus although it has generally been assumed that that organism was too sensitive to desiccation to live very long in fine particles.

I.—EXPERIMENTS ON THE VIABILITY OF THE PNEUMOCOCCUS IN LARGE MASSES OF SPUTUM.

EXPERIMENT I.—The following tests were made to determine the viability of the pneumococcus in sputum when kept moist and at room temperatures and also when kept at 0° C.

TABLE I.

TESTS WITH MOIST SPUTUM.

Day of Test.		1	5	10	15	20	30	42	60
I. Thick, mucous sputum from 3d day of pneumonia	22° C.	+	+	o	o	o	o	o	o
	0° C.	+	+	+	+	+	+	+	o
II. Thick, rusty sputum from 3d day of disease....	22° C.	+	+	+	o	o	o	o	o
	0° C.	+	+	+	+	+	+	o	o
III. Thin, fluid sputum from 5th day of disease.....	20° C.	+	+	+	+	+	o	o	o
	0° C.	+	+	+	+	+	o	o	o
IV. Thin, yellowish sputum after crisis.....	20° C.	+	+	o	o	o	o	o	o
	0° C.	+	+	+	+	+	+	+	o
V. Thick, yellowish sputum from 8th day of disease...	20° C.	+	+	+	+	o	o	o	o
	0° C.	+	+	+	+	+	+	o	o

The positive marks mean that the pneumococcus was either isolated from the sputum by culture, or, especially after the 5th day, that the subcutaneous injection of from one fourth to one fifth of a cubic centimeter of the undiluted sputum was fatal to a mouse. No result was considered as positive unless capsulated, Gram-positive organisms could be isolated from the blood of the animal, and unless the coccus fermented inulin after plating out on chest-serum agar. Occasionally by the use of very large quantities of sputum (0.5 to 1.5 c.c.) it was possible to kill mice up to fifty days, but often only one animal out of three died, showing that only a few organisms remained alive.

It will be seen from the table that the life of the pneumococcus in fresh, moist sputum at room temperatures is rarely over two weeks. The specimens were kept in the dark in order to compare them directly with those at 0° C., which were of necessity inclosed in a cold-storage box. Two specimens kept in strong diffuse daylight lost virulence for mice in less than five days.

The rapid death of the organisms in sputum as compared to chest-serum is possibly due to the bactericidal action of the mucus of the sputum.

EXPERIMENT II.—Tests were also made by drying sputum in Petri dishes at room temperatures. Some of the specimens were kept in a dark, dry spot, others were exposed to diffuse daylight in a room facing the south, others were exposed to full sunlight. Fragments of the dry crust of sputum were then removed, rubbed up in sterile bouillon, and inoculated into mice. Other specimens

were finely powdered in a mortar with a few fragments of glass, and the dust exposed to daylight or direct sunlight. The results are as follows:

TABLE II.

TESTS WITH DRIED SPUTUM.

Day of Test.	1	4	8	12	20	30	40	60	70	80
Sputum kept in dark:										
No. I. Thin, watery.....	+	+	+	+	+	+	+	o	o	o
" II. Thick, mucous....	+	+	+	+	+	o	o	o	o	o
Sputum exposed to diffuse light:										
No. I. Thin, watery ...	+	+	+	+	+	+	o	o	o	o
" II. Thick, yellow, mucous.....	+	+	+	+	+	o	o	o	o	o
" III. Thick and rusty.	+	+	+	+	+	+	+	o	o	o
Sputum dried over calcium chloride in daylight:										
No. I. Thin, watery.....	+	+	+	+	+	+	+	+	o	o
" II. Thick, mucous ...	+	+	+	+	+	+	+	+	+	o

The specimens of sputum dried over calcium chloride retained their virulence for mice for a slightly longer period than those exposed to the air. This is probably due to the very complete and prompt drying which takes place. The specimens exposed to the air never dry completely, and the amount of moisture retained varies from day to day in accord with the atmospheric changes.

TABLE III.

TESTS WITH DRIED AND PULVERIZED SPUTUM.

Hours of Test.	1	2	4	8	12	24	36	48
Sputum dried and exposed to sunlight:								
No. I.	+	+	+	o	o	o	o	o
" II.	+	o	o	o	o	o	o	o
" III.	+	+	o	o	o	o	o	o
Sputum finely powdered and kept in dark:								
No. I.	+	+	o	o	o	o	o	o
" II.	+	+	+	o	o	o	o	o
Exposed to diffuse light:								
No. I.	+	o	o	o	o	o	o	o
" II.	+	o	o	o	o	o	o	o
Sputum finely powdered and exposed to direct sunlight:								
No. I.	o	o	o	o	o	o	o	o
" II.	o	o	o	o	o	o	o	o

It is evident from the table that the exposure of the pneumococcus to sunlight results in the prompt death of the organism. The mere powdering of the sputum also destroys the pneumococcus, a phenomenon probably due to the rapid and complete drying which takes place. The action of even diffuse daylight in hastening the death of the organism is evident from the table. Exposure of the powder to sunlight effects an even more rapid destruction, there being probably three factors in the process. One is the formation of oxidizing agents, probably hydrogen peroxide, by the action of the sun's rays upon the traces of moisture remaining in the sputum,[34] a second the rapid drying which takes place, and a third, the destructive action of the chemical portion of the sun's rays.

Experiment III.—Sputum was spread on fragments of sterile wood, and tin, and on woollen and cotton cloth. The specimens were allowed to dry, and were kept either in diffuse daylight or sunlight. The life of the organism was about the same on wood and tin as on glass. On cloth several tests gave a slightly longer life, the sputum being virulent for mice after sixty days. This is explained by the penetration of the cloth which takes place when soaked with sputum, the fiber of the cloth protecting the organism from light and the layer of sputum formed being thicker than on a flat surface. This effect was more marked in those fragments exposed to sunlight, one piece of woollen cloth being virulent to mice after twelve hours' exposure, about six hours being given on two successive days in May. The death of the bacteria occurred on two hours' further exposure.

II.—VIABILITY OF THE PNEUMOCOCCUS IN FINE SPRAYED PARTICLES.

Technique.—In order to spray sputum and other fluids containing pathogenic bacteria and to collect the finer particles, it is necessary to conduct the operation in an air-tight chamber, to avoid contamination of the laboratory and infection of the operator.

The apparatus employed by the writer was modelled upon the one described by Kirstein,[35] with some slight modifications. The box was constructed of seven-eighths inch white wood lumber with internal measurements of 38 cm. in depth, 35 cm. in width, and 152 cm. in length. At one end were perforations

[34] Bie, *Mitth. aus Finsens Med. Lysinstitut*, 1905, Neuntes Heft, p. 5.
[35] *Zeit. f. Hyg.*, 1900, xxxv, 145.

for the insertion of the tip of the spraying apparatus and apertures to permit of the escape of air driven into the chamber while spraying the sputum. In order to prevent direct carrying of particles the full length of the chamber and the deposition of the organisms in coarse masses upon the Petri dishes or other substances used to collect the spray, two baffle plates were placed about the middle of the chamber, 22 cm. apart. These plates were of glass and measured 28 by 35 cm. They were held in place by narrow strips of wood nailed on the inner side of the box, and further secured by putty and a layer of enamel paint. The plate nearer the spraying apparatus was so placed that its upper portion was in contact with the lid of the box. The plate farther from the spraying apparatus was in contact with the floor of the box, leaving a space of 10 cm. between its upper edge and the lid (see Fig. 1). It was thus impossible for particles from the spray to pass directly from one end of the box to the other. The coarser masses strike the first plate and adhere to it. Only the finely suspended particles pass over the top of the second plate, and this only when a current of air is drawn through the apparatus (see Experiment IV).

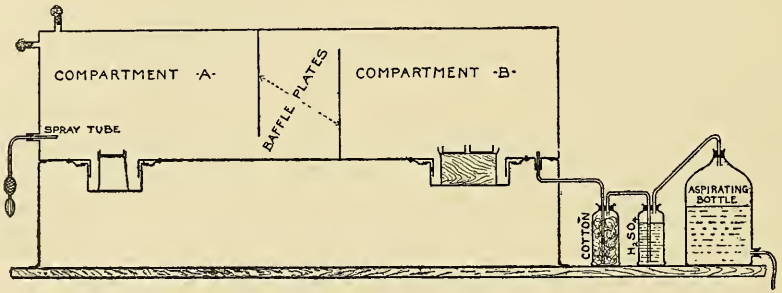


FIG 1 Diagram of Box for Spraying Pathogenic Bacteria.

In order to collect the sprayed particles, three apertures were made at the bottom of the box, two of which were circular, measuring 13 cm. in diameter; the third was rectangular and measured 26 by 14 cm. These openings had a tin collar inserted in them extending about 10 cm. below the bottom of the box. These collars were rendered air-tight by white lead. Each opening was closed during the experiment by placing under it a dish some 3 or 4 cm. larger in diameter than the collar, the dish being filled with 1:1000 mercuric chloride solution, thus making a water seal. The dishes were held in position by the use of small cupboard buttons which could be swung into place under the rim of the dish. Before the spraying was commenced, suitable receptacles for catching the spray, such as Petri dishes, either dry or containing culture media, or fragments of sterilized cloth, wood, tin, etc., were placed on small stands which rested on the bottom of the dish. For the smaller apertures in the first compartment these stands were ordinary drinking-glasses which were inverted and the upper end surrounded by a strip of half-inch surgical adhesive plaster. The Petri dish placed on this adhered quite firmly, and there was no danger of its falling off during the process of removal, even though the glass was consider-

ably tipped. In the larger rectangular opening the stand was made of half-inch pine board with four nails for legs, surrounded by a collar of adhesive plaster. This collar retained the plates in position and prevented their shifting during insertion or removal of the stand. The surface of the Petri plate when inserted was approximately level with the bottom of the spraying-box.

In order to render the inside of the box air-tight and waterproof, the corners were filled with putty and the inside was given three coats of thick enamel bath-tub paint. The lid was held in position by one-eighth inch steel wires which passed from a turn buckle fastened to the lid on one side, underneath the box and over a wooden brace to a turn buckle on the opposite side. The turn buckles could be screwed tight, thus holding the lid firmly in position. In order to make a suitable seal, the upper edge of the box was smeared with a thick layer of paste sold commercially as anti-phlogistine. This was found to be better than putty or white lead, as it did not set, but remained moist and somewhat pliable for a period of nearly two months. At the end of the box farthest from the spraying apparatus was an aperture similar to those in the spraying end of the box. A suitable opening was made by boring a hole from the outside of the box, about 22 mm. in diameter. A collar, about 3 mm. in width, made of wood, was left on the inside of this opening. Short pieces of brass tubing were heated over a Bunsen flame, smeared with rosin, and quickly inserted in the holes, into which they fitted snugly, the collar of wood which was left insuring a firm seat. As soon as the rosin cooled, an air-tight joint was obtained. Four of these apertures were made: one for the insertion of the spray tube, one for aspiration of the air current, two for egress of the air forced in by the pressure apparatus. The spray was produced by a long glass spray tube which was inserted through one of the openings and tightly packed in position with absorbent cotton. After the spraying was completed, these tubes could be easily sterilized by boiling in one per cent. sodium carbonate solution. The spraying was done by means of compressed air, the pressures used varying from five pounds or less to the square inch to forty pounds to the square inch. It was found necessary to use a higher pressure in the case of thick, mucous sputum than for thin, watery sputum. By this means suitable quantities of thick sputum, in many instances 30 to 40 c.c., could be atomized during the course of ten minutes. Before beginning the experiments the box was tested by closing all the apertures with corks and inserting a water manometer in one of the openings and forcing in air through another. A pressure of three inches of water was sustained for fifteen minutes, showing that the box was air-tight for this pressure. Higher than this it was impossible to go because the water-seals would have been forced by the pressure. As it was, some difficulty was experienced in spraying into the apparatus unless air was being drawn out at the same time, because of the escape of bubbles of air through the water-seals. It would be advisable, therefore, to modify the apparatus and make the seals somewhat deeper; possibly 15 cm. would be better than the 10 cm. used. Air was drawn through the box by means of an aspirating bottle graduated in liters. In order to catch any bacteria and prevent their entry into the aspirator, a bottle was inserted between the box and the aspirator containing tightly packed absorbent cotton, and a second containing sulphuric acid through which the aspirated air was forced to bubble. The rate of aspiration could be measured

by timing the rate of outflow from the bottle. The speed at which the particles were carried from the chamber nearer the spraying apparatus, which may be for convenience termed A, into the chamber farther from the spraying, called B, could be determined as follows. (Fig. 2.)

The distance between the two baffle plates being 22 cm., their height 28 cm., the length of the hypothenuse would be about 36 cm. The diameter of the channel is then approximately 6 cm. The cubic contents of this channel from the lower aperture of the first baffle plate to the upper aperture of the second would be 36 by 35 by 6 cm. This is approximately 7560 c.cm. If this amount of air is aspirated from the farther end of the box in one minute, the velocity in the channel will be 6 mm. per second, which is near the lowest limit of air speed which will move very fine particles. Air speeds, therefore, were used in these experiments of from 2 to 10 mm. per second.

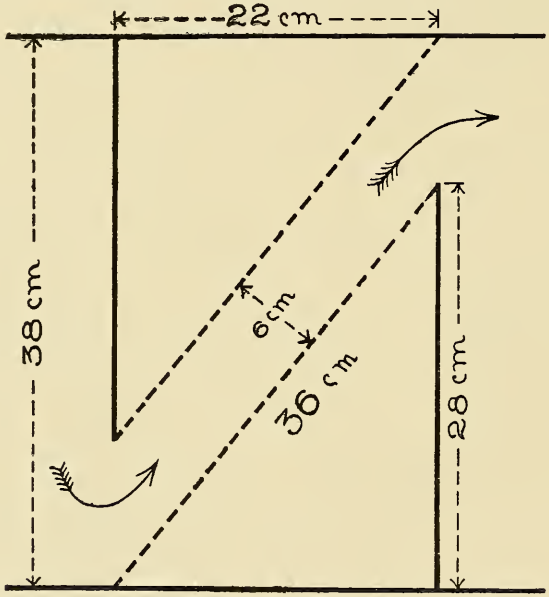


FIG. 2. Diagram of Dimensions and Course of Air Current in Spraying Box.

EXPERIMENT IV.—A preliminary experiment made with bouillon cultures of B. prodigiosus showed the box to be tight and that no bacteria passed the baffle plates unless a current of air was drawn through the box.

Sputum was then obtained from a case of acute lobar pneumonia at about the third day of the disease. Morphological examination showed numerous diplococci in nearly pure culture. They were positive to Gram, but no capsules were demonstrable. The sputum was plated on chest-serum agar and diplococci isolated which were Gram positive, had well marked capsules, fermented inulin, and killed a mouse in three days, capsulated cocci being found in the heart's blood.

Ten cubic centimeters of this thick sputum were sprayed in the box, using 40 pounds air pressure. During the spraying and for some time after, a slow current of air was drawn through the apparatus at a rate of about 0.2 mm. per second. Cover-slips exposed in the second compartment during spraying showed numerous particles derived from the spray, some of which contained pneumococci or the other bacteria of the sputum. A current of 0.2 mm. per second is therefore capable of transporting spray-carrying bacteria for a distance of at least one meter.

After the spray had been stopped, covers were exposed every fifteen minutes for two hours. At the end of an hour most of the particles carrying bacteria had settled. Covers exposed after ninety minutes had elapsed showed no bacteria, only very small particles of mucus taking a blue stain with gentian violet. This shows that the bacteria may be assumed to settle 38 cm. in from sixty to ninety minutes.

In order to study the settling of the particles more conveniently and to determine the length of time for which sprayed sputum particles can remain in suspension, ten cubic centimeters of this very thick sputum were sprayed at 40 pounds pressure into a tall aspirating jar about 45 cm. in height, the air contents of which had been cleansed of dust by aspiration through a thick cotton plug. A jar was used for this preliminary experiment instead of the box just described, because of the ease with which suspended particles could be rendered visible by a strong beam of light. A thick fog of the sprayed particles was produced which remained suspended for sixteen hours and could be rendered easily visible by passing a beam of light from an electric arc. At the end of twenty-four hours only a few fine particles could be seen on concentrating the light with a lens. No bacteria were deposited on cover-glasses or culture plates after the jar had stood for two hours.

In order to determine whether the fine spray particles, which remained a long time in suspension after spraying a broth culture, contained bacteria, the jar was filled with spray from a bouillon culture of B. prodigiosus, and after standing one hour the plug was removed from the upper end and the bottle was reversed and allowed to rest on the mouth of a large battery jar. Under the mouth was placed an agar covered Petri dish. At the end of one hour the dish was removed, covered, and allowed to remain at room temperature for several days. Abundant growth took place.

In a repetition of this experiment growth was obtained by allowing the fog to settle on plates exposed at the end of one hour and thirty minutes, but no growth was obtained after two hours, nor after four and six hours. This agrees with the results obtained by Stern,[36] who states that ordinary dust particles settle in still air in from one hour and a half to three hours, and can only be kept afloat by air currents of from ten to thirty millimeters per second. Very fine particles still containing bacteria can

[36] *Zeit. f. Hyg.*, 1889, vii, 44.

be transported laterally by a current of 0.2 mm. per second, and kept afloat by a current of from 0.3 to 0.4 mm. per second.[37] The fog made evident by the light beam after a period of from five to six hours is probably composed of dried salts and albumin or mucus particles, and does not contain bacteria.

Air currents which cause the movements of these very fine particles have been shown to be of much less velocity than those which occur in well ventilated rooms where the motion is from 1 to 2 mm. per second. Air at ordinary temperatures does not produce a perceptible draught until its velocity reaches 10 cm. per second. In unventilated rooms the current is less than 0.6 mm.

It is therefore possible for spray particles containing the pneumococcus to float in the air of an unventilated room for some three hours, if we assume the rate of fall as determined by the experiments to be at least 30 cm. per hour and the head of the patient to be about one meter from the floor. With air currents of very slight intensity, however, the finer particles may be carried for considerable distances. Many of these fine droplets do not contain bacteria, so that the practical danger from a patient with pneumonia is less than appears from tests under artificial conditions. The coarse particles containing many bacteria fall rapidly, and in the case of the pneumococcus, as will be seen later, many of the suspended organisms lose their vitality in the course of one or at most two hours.

Experiment V.—In order to avoid the use of mixed cultures such as would be obtained from sputum, a pleuritic fluid containing enormous numbers of pneumococci was also employed in the studies. This fluid was obtained by injecting small amounts of sputum into the right pleural cavity of large rabbits. The injection is easily made by passing a fine needle through one of the intercostal spaces on the lateral aspect of the thorax. The animals usually die in two to three days, and if the thorax is carefully opened from 10 to 50 c.c. of clear or slightly bloody fluid can be obtained. Usually both pleuræ and the pericardial sac contained fluid.[38]

Ten cubic centimeters of the pleuritic fluid were sprayed, and at the same time sixteen liters of air were removed at such a rate that the velocity between the baffle plates was 2 mm. per second. The control plate in Chamber A was

[37] Flügge, *Zeit. f. Hyg.*, 1897, xxv, 193.

[38] This method of obtaining a fluid rich in pneumococci was suggested to me by Dr. A. B. Wadsworth. The organisms remain alive in the serum for a long time at 0° C.

removed after thirty minutes and was found to be slightly moist. Bouillon was poured on the surface and rubbed up with a platinum needle. Loops from the bouillon were then transferred to chest-serum agar. An abundant growth of pneumococci was obtained. Plates exposed in Chamber B were removed at the end of an hour and a fresh set inserted. Cover slips which had been exposed during the same time showed numerous capsulated cocci. Bouillon was poured over one plate, rubbed up with a platinum spatula, and injected into a mouse. The animal died from pneumococcus infection. Other plates from Chamber B were exposed one, two, and three and a half hours to diffuse daylight. At the end of this time mice were inoculated from the two-hour plates, and two rabbits [39] were injected in the ear vein with an emulsion from two three-and-a-half-hour plates. One mouse died twenty days later, but no pneumococci could be recovered. The other lived for two months. The rabbits did not die. A third plate after three and a half hours was covered with chest-serum agar, but no growth was obtained. Another plate from Compartment B was exposed three hours to sunlight on a slightly overcast day. Plates were made after emulsifying with bouillon and three mice injected. There was no growth on plates. One mouse died twelve days later, but no pneumococci could be demonstrated. The others did not die. Another plate was dried over calcium chloride for three hours in diffuse light. Plate cultures and animal inoculations were negative.

The results of this experiment may be considered as showing that spraying a thick albuminous fluid containing pneumococci and allowing the fine spray to dry on glass is fatal to the organisms in a very short time. If drying is prevented by collecting the particles as they fall on moist chest-serum agar, growth will be obtained if the bacteria have not been in suspension over ninety minutes. A fluid of the type used corresponds pretty closely to the thin, serous sputum of certain cases of pneumonia.

Experiment VI.—Twenty-five cubic centimeters of the thick sputum used in Experiment IV were sprayed in the box during ten minutes, using an air current of about 6 mm. per second. Control plates from Compartment A were positive. Plates from Compartment B removed immediately were negative to mice, and cultures gave only staphylococci. Cover-glasses showed numerous drops varying from three to fifteen micra in diameter. The larger drops frequently contained two or three diplococci.

One hour after spraying, cover-glasses were placed in Compartment B and allowed to remain fifteen hours. These showed only a few bacteria, less than one per square centimeter. There were, however, many masses of mucus which had fallen on the slide, most of which did not contain bacteria. No free organisms were found, all were surrounded by more or less mucus.

[39] It was thought, inasmuch as the pneumococci used had been adapted to rabbits, that these animals might be more susceptible to infection than mice.

Chest-serum-agar plates, exposed in Compartment B for a period beginning one hour after the spraying was finished, gave abundant growth of staphylococci, but mice injected with an emulsion showed no pneumococci. Some plates remained in Compartment B for a number of days, but no pneumococci could be demonstrated. Dry plates remained ten days, and when covered with agar gave numerous colonies of Staphylococcus pyogenes aureus. Mice which were injected died, but no pneumococci could be isolated. Plates from this spraying were kept in the dark and their contents injected into mice at various intervals. Some of the animals died, but no pneumococci could be obtained.

A plate dried over calcium chloride for fifteen hours in the dark gave no pneumococci, but only staphylococci.

The results of the experiment show the rapidity with which the pneumococcus dies when sprayed in fine particles and allowed to dry. The drying seems to be an important factor, for if, as is shown in Experiment V, the bacteria are caught on moist media, a growth will be obtained. As a rule also we must assume that there are other factors at work, for the viability of the organisms after spraying sputum is certainly less than that after spraying rabbit chest-serum. The action of the mucus must be considered and possibly also the osmotic relations of the organism to the sputum may affect the pneumococcus unfavorably. It is also not impossible that a considerable proportion of the pneumococci in sputum from the later stages of the disease are not viable.

Experiment VII.—Forty cubic centimeters of fresh, thin, serous sputum, from the sixth day of the disease, one loop of which was capable of killing a mouse in forty-eight hours, were sprayed for one hour with an air current of 10 mm. per second. The control plate from Chamber A killed a mouse in forty-eight hours. Pneumococci were isolated which were positive to Gram, were capsulated, and fermented inulin. The plate was dry when it was removed from the compartment.

Plates from Chamber B, removed at the end of spraying and found to be dry, were washed with bouillon and the washings injected into two mice. One died, but no pneumococci were found; the other lived a month. Washings from these plates were sown on the surface of chest-serum agar in order to avoid the inhibiting action of the anaërobic conditions which exist under a layer of agar. Staphylococci and other unidentified organisms were obtained, but no pneumococci.

Plates dried over calcium chloride were also negative as regards pneumococci. These experiments were repeated with sputa from different cases and at different times of the disease, but the results were practically the same.

It is evident that finely sprayed sputum contains no viable pneumococci after drying on glass for one hour. The positive results occasionally obtained from the Control plates in compartment A may be explained by the thick layer of sputum which is deposited and prevents complete desiccation. The results obtained by using thin, serous sputum do not vary from those obtained when thick, mucous sputum was sprayed, although differences appear when the sputa are dried in bulk.

Experiment VIII.—The technique was varied slightly so as to transfer large numbers of the organisms and thus to keep them moist. About 100 c.c. of pleuritic fluid were sprayed, with an air current of 10 mm. per second. Fifteen minutes after spraying was completed the plates in Compartment B were removed. They were still moist. A mouse injected with an emulsion of the deposit died with pneumococci in the heart's blood. A plate was exposed to sunshine for twenty minutes, during which time it dried. A mouse injected did not die. A plate of the same series was dried over calcium chloride for thirty minutes. One-half was injected into a mouse which died with pneumococcus sepsis. The plate was dried thirty minutes more; mouse died with pneumococci in the heart's blood. A plate from the same series was dried two hours over calcium chloride; a mouse injected did not die. A broth culture was made from this plate and showed pneumococci which killed a mouse (see results obtained by Ottolenghi). Another plate was dried for three hours and a mouse injected and a broth culture made. Broth was negative and the mouse remained alive. Plates dried in air for one hour were positive; for one and a half and two hours, negative.

It is possible that when only a small quantity of fluid is sprayed there are not enough virulent pneumococci left after drying to kill the experimental animal. A certain minimum dose seems necessary to kill even as susceptible an animal as a white mouse. A larger quantity of fluid was therefore sprayed in this test, and as shown by the slightly longer life of the pneumococci as compared to Experiments V, VI, and VII, the quantity exerts some influence. The conditions approach those which occur in drying sputum in bulk (see Table II) where the life of the organism is considerably prolonged.

During this test fragments of sterilized woollen and cotton cloth, tin, and wood were exposed in Compartment B. They were removed, allowed to dry in the air for thirty minutes, and scrapings from the surface tested. The organisms on the tin and wood were dead, those on the cloth were alive, but died on drying for thirty minutes longer.

Experiment IX.—A number of observers have thought that the pneumococcus in sputum is rapidly destroyed by the bactericidal action of the mucus of the sputum. Such action has been shown to take place with nasal and uterine mucus and pure mucin.[40]

[40] Wurtz and Lemoyez, *Compt. rend. de la Soc. de Biol.*, 1894; Arloing, *Jour. de phys. et de path. gén.*, 1902, iv, 291 (Bibliography).

(*a*) This was tested by keeping a thick, mucous sputum at 0° C., as recorded in Experiment I. As the pneumococcus dies on culture media or in rabbit serum in a few days unless kept at 0° C., it was thought that a better differentiation could be obtained by working at the lower temperature and in the dark. When first collected the specimens killed mice in doses of a few cubic millimeters in forty-eight hours. After fifteen days at 0° C., a much larger amount of sputum was required to kill a mouse of about the same size as that used during the first experiment. At the end of six weeks mice often could be killed only by doses of a cubic centimeter of pure sputum, while one specimen was no longer virulent after twenty days. Evidently a large number of the pneumococci die in two weeks when kept in moist sputum.

As it is well established that the pneumococcus remains alive for a long period when kept in serum mixtures at 0° C., a combination of this fluid with sputum should retain its virulence as long as pure sputum unless some bactericidal agent is present in the sputum. Such a mixture was therefore made and kept in Petri dishes. The sputa used were the same as in Table I. The results were as follows:

TABLE IV.

TESTS WITH SPUTUM-CHEST-SERUM MIXTURES KEPT AT 0° C.

Day of Test.	1	5	10	15	20	30	42	60
Sputum No. I.: +serum containing pneumococci.	+	+	+	+	+	+	+	o
Sputum No. II.: +serum containing pneumococci.	+	+	+	+	+	+	o	o
Sputum No. III.: +serum containing pneumococci.	+	+	+	+	+	+	+	o
Sputum No. IV.: +serum containing pneumococci.	+	+	+	+	+	+	+	o
Sputum No. V.: +serum containing pneumococci.	+	+	+	+	+	o	o	o
Serum alone.....	+	+	+	+	+	+	+	+

The table shows that the serum-sputum mixtures do not retain their virulence for mice much longer than the original unmixed sputum as given in Table I. In two cases, however, that of sputum No. III and No. IV, virulent pneumococci were still present at the end of six weeks, while the pure sputum was non-virulent after three weeks' preservation. This difference is possibly due to the fact that but little mucus was present in the sputum. The practical importance of these findings is that the

thin, serous sputa are likely to retain their infectious qualities somewhat longer than the thick, mucous specimens, and as the thin sputa are most easily sprayed during coughing, special care should be taken to avoid contact infections.

In order to determine the action of the mucus during spraying and after drying of the spray particles, the following experiment was planned.

(*b*) Specimens of sputa Nos. VI and VII were mixed with an equal quantity of rabbit chest-serum rich in pneumococci. Mice injected with the mixture died promptly of pneumococcus infection. The specimens were kept on ice in the dark, and in diffuse daylight at room temperature. The results were as follows:

TABLE V.

TESTS WITH SPRAYED SPUTUM-CHEST-SERUM MIXTURE.

		Sprayed after				
		2 days.	4 days.	8 days.	15 days.	20 days.
Sputum VI.: + serum on ice in dark. Thin, serous sputum.	Plates removed from Comp. B. immediately.....	+	+	+	+	+
	Dried in air for 30 min.....	+	o	o	—	—
	" " sunlight for 30 min.	o	o	o	—	—
Sputum VI.: + serum in dark at room temp.	Same conditions as above.	+	o	—	—	—
		o	o	—	—	—
		o	o	—	—	—
Sputum VII.: + serum on ice. Thick mucous sputum.	Same conditions as above.	+	+	o	—	—
		o	o	o	—	—
		o	o	o	—	—
Sputum VII.: + serum at room temp. (18°–22° C.).	Same conditions as above.	o	o	o	—	—
		o	o	o	—	—
		o	o	o	—	—
Sputum VI.: Without admixture on ice.	Same conditions as above.	+	+	+	o	—
		o	o	o	—	—
		o	o	o		
Sputum VII.: Without admixture in dark at room temp.	Same conditions as above.	+	o	o		
		o	o	o		
		o	o	o		
Chest-serum on ice....	.....	+	+	+	+	+
Chest-serum in light...	.....	+	+	+	o	—

The results of the experiments show that pneumococci die off in mucous sputum more rapidly than they do in a serum mixture and that this action is probably due to the mucus present. The pure serum used in this test preserved its virulence for weeks when kept on ice, and for eight days in diffuse light.

Experiment X.—In order to determine whether the rapid death of the sprayed organisms is due to the drying which takes place while they are suspended in the air or after they are deposited on the glass plates or other dry substances used to collect them, the following variation was made in the test.

Thirty cubic centimeters of pleuritic fluid were sprayed, using an air current of 10 mm. per second. The contents of the control plates removed at the end of the spraying killed mice in two days and gave an abundant growth on serum-agar. Plates of serum-agar were inserted in Compartment B at the completion of the spraying and the air current was continued for thirty minutes. These plates were removed in thirty minutes and a second set of serum-agar plates was substituted. These were also removed in thirty minutes and a fresh set substituted.

On the first group of plates there was an abundant growth of pneumococci which killed mice—i. e., had lost none of their virulence. The second set of plates showed about twenty colonies each. These were, of course, derived from bacteria which had been in suspension for at least thirty minutes. The third set inserted at the end of an hour after the spraying had ceased and allowed to remain for three and a half hours, showed one or two colonies of pneumococci. Cover-glasses inserted at the same time showed no demonstrable pneumococci after a long search and only small masses of deposited spray. It is evident that practically all the bacteria had settled out from a height of 38 cm. in an hour's time, and that those in suspension for that time were still alive, probably owing to their being protected from complete desiccation by the inspissated serum surrounding them. In order to extend the time during which the organisms could be suspended in the air, a sputum-chest-serum mixture was sprayed into a tall aspirating jar some 45 cm. in height. As the rate of fall in still air of fine particles containing pneumococci is about 40 cm. per hour, the jar was inverted every fifteen minutes for two hours, during which time it was exposed to diffuse light. It was then fixed mouth downward over a Petri dish containing chest-serum agar and was left for six hours in the dark. Numerous colonies of Staphylococcus pyogenes aureus developed, but none of the pneumococcus.

As shown above, the organism is alive after an hour's suspension. A second test showed that only a few pneumococci survive for ninety minutes when suspended in a fine spray in diffuse light. Such a fact is of the greatest importance from a point of view of the hygiene of those in close contact with persons suffering with pneumonic infections. It demonstrates the necessity of an abundant air supply to dilute the cloud of organisms which sur-

round a patient with a severe cough. A repetition of the same test allowing the jar to stand in direct sunlight for fifteen and thirty minutes, and then removing it to a dark room to permit the organisms to settle, showed in a very striking manner the value of sunlight as a disinfectant. Only a few colonies of the pneumococcus were obtained after fifteen minutes, and none at the end of half an hour.

SUMMARY AND CONCLUSIONS.

I. In moist sputum kept in the dark at room temperatures the average life of the pneumococcus is eleven days, though considerable variations may be noted in different specimens of sputum.

In the same sputum kept at 0° C. the average life of the organism is thirty-five days.

In sputum kept at room temperature and in a strong light the pneumococcus lives less than five days.

II. In dried sputum (*a*) in the dark the pneumococcus lives on an average thirty-five days; (*b*) in diffuse light, thirty days; (*c*) in sunlight, less than four hours.

III. In powdered sputum even when kept in the dark the death of the pneumococcus takes place in from one to four hours. When exposed to sunlight death occurs within an hour.

IV. No important differences were noted in the life of the pneumococcus when dried on glass, tin, or wood. On cloth the life was usually slightly longer than on non-absorbing surfaces.

V. Sprayed sputum particles remain in suspension for twenty-four hours, but all masses of a size sufficient to contain bacteria settle at a rate of about 40 cm. per hour.

VI. When sputum containing pneumococci is sprayed the organisms rarely survive for more than an hour, and often die in less time. The substance upon which the particles fall makes but little difference in the life of the organism. On cloth a slight prolongation is occasionally noted, due perhaps to the slow drying.

VII. The mucus of the sputum exerts a destructive action on the pneumococcus.

VIII. Exposure of bacterial spray to sunlight while in suspension results in the destruction of the pneumococcus within half an hour.

IX. The conclusions of practical importance which can be drawn from the facts given in this paper are as follows:—

A. The life of the pneumococcus in moist sputum is of considerable duration, the average period being less than two weeks unless the material is exposed to direct sunlight. But as such sputum does not give off bacteria even when exposed to strong currents of air, it may be considered as innocuous except to persons handling clothes, bedding, etc., which have recently been contaminated. Under ordinary conditions, however, this sputum dries in the course of a few hours or days. The dried masses retain their virulence for a long time, and if deposited on the floor or on the bedding of the patient may be powdered mechanically, and sweeping, dusting, or brushing the contaminated articles will distribute pneumococci in the air. Fortunately, however, the organisms in the sputum do not remain long in suspension and die off rapidly under the action of light and desiccation. In sunlight or diffuse daylight the bacteria in such powder die within an hour, and in about four hours if kept in the dark. The danger of infection from powdered sputum may, therefore, be avoided by ample illumination and ventilation of the sick-room in order to destroy or dilute the bacteria, and by the avoidance of dry sweeping or dusting. Articles which may be contaminated and which cannot be cleaned by cloths dampened in a suitable disinfectant should be removed from the patient's vicinity.

B. When a person suffering from a pneumococcus infection coughs, sneezes, expectorates, or talks, particles of sputum or saliva are expelled from the mouth which may contain virulent pneumococci. Such particles remain suspended in the air for a number of hours if the ventilation of the room is good. They may be inhaled by persons in the vicinity of the patient, or they may be deposited upon various articles in the room. Whether suspended in the air or dried on surrounding objects, the writer's studies show that they become harmless in a very short time,

about an hour and a half being the extreme limit, while many of the pneumococci in the spray perish in a few minutes, especially if exposed to strong light.

In the light of these experiments the risk of infection from the pneumococcus is largely confined to those in direct contact with the person whose excreta contain the organism.

The writer wishes to acknowledge his obligations to Prof. T. Mitchell Prudden for many helpful suggestions made during the course of this study.

A NOTE UPON THE GROWTH OF PNEUMOCOCCI AND STREPTOCOCCI IN BLOOD SERUM.

By WARFIELD T. LONGCOPE, M.D.

Director of the Ayer Clinical Laboratory of the Pennsylvania Hospital.

During the investigations described in another paper defibrinated blood and blood serum were frequently used, either alone or mixed with agar, as media upon which to grow pneumococci and streptococci. It was immediately noted that the organisms grew very differently in serum obtained from different individuals. The variations were so marked and the results obtained so striking that it was decided to study the matter more carefully. To this end the growth of pneumococci and streptococci was studied in the blood serum of thirty-two individuals. Besides the human sera, horse's serum, calf's serum, and rabbit's serum were used.

In the researches conducted by Besançon and Griffon,[1] Huber,[2] Neufeld,[3] and Wadsworth[4] upon the agglutination of pneumococci by the serum of pneumonia patients and of immunized animals, certain changes besides the actual agglutination were noted in the bacteria and serum. Huber in particular calls attention to the heavy precipitate formed by growing pneumococci in pneumonic serum, a phenomenon which did not occur in the serum from normal individuals, when used as a culture medium for pneumococci. Neufeld describes swelling of the capsules of the pneumococci when treated with the serum from cases of pneumonia or from immunized animals. Wadsworth has confirmed these observations. Recently Rosenow[5] has called attention to another very interesting result of the

[1] *Annal. de l'Inst. Pasteur*, 1900, xiv, 449.
[2] *Cent. f. inn. Med.*, 1902, 417.
[3] *Zeit. f. Hygiene u. Infectionskrank.*, 1902, xl, 54.
[4] *Journal of Med. Research*, 1903, x, 228.
[5] *Journal of Infectious Diseases*, 1904, ii, 280.

growth of pneumococci in pneumonic serum. After two or three days' growth acid is formed in the serum. This does not occur when the serum from normal individuals is used. Scarlet-fever serum, however, did yield acid. The rich precipitate which forms in the serum after the growth of pneumococci is due, at least in part, as Rosenow thinks, to the action of the acid upon the serum, and is not entirely formed, as was thought by Huber and Wadsworth, by masses of agglutinated pneumococci.

The pneumococci in the present study were obtained both from the saliva of normal individuals and from the consolidated lungs in cases of lobar pneumonia. The streptococci were procured from the saliva of normal individuals and from pathological conditions.

The serum was obtained from persons suffering from various diseases. In all there were fifteen cases of pneumonia, five of chronic nephritis and uræmia, two of general streptococcus infection, one case of gonorrhœal endocarditis (gonococcus recovered in blood culture), three cases of tuberculosis, one case of acute articular rheumatism, four cases in which the diagnosis was not absolutely certain, and one normal individual. To collect the serum, about 20 c.c. of blood were drawn from the arm vein and allowed to clot in a cool place; after from twenty-four to forty-eight hours the serum was drawn off. The properties of the serum, so far as the growth of the pneumococci and streptococci was concerned, did not alter with age. The same reactions were seen in the serum twenty-four or forty-eight hours and several weeks after the blood was drawn. From 2 to 5 c.c. of serum were used as a culture medium.

In the normal serum the pneumococci produced a slight cloud, the streptococci a granular sediment. The serum remained alkaline. In the serum from the cases of pneumonia the growth of the pneumococcus was extremely interesting. After twenty-four hours there was a diffuse cloud, often with a heavy, whitish precipitate, and the fluid appeared a little thick. The reaction varied, with litmus as an indicator, from neutral to well-marked acidity. In hanging-drop preparations small clumps of highly refractile bodies were seen. They were two or three times the

size of pneumococci but had much the same morphology and appeared as pneumococci with greatly swollen capsules. Besides these swollen clumps there were many single cocci which were not swollen and many minute granules, the morphology of which could not be definitely determined. Preparations stained with the usual dyes showed well preserved pneumococci with capsules. The clumps of swollen refractile bodies stained poorly and only occasionally could they be identified as bacteria.

With increased growth the cloud and thickening progressed until after from six to eight days the fluid was opaque, white, of almost semi-solid consistency, and looked much like highly albuminous urine after treatment with heat and acetic acid. During the growth the acidity steadily increased. At the end of from six to eight days' growth, it usually required 0.3 to 0.7 c.c. of $\frac{1}{10}$ N. sodium hydroxide to neutralize 1 c.c. of the serum, when litmus was used as an indicator. With continued growth the masses of swollen refractile bodies increased greatly in size and numbers; the remainder of the cloud seemed to be made up of fine granules. With the various dyes only a few pneumococci took the stain. The streptococci grew in an entirely different manner. They usually produced no cloud but a fine granular sediment, often grew very poorly, and never gave rise to an acid reaction.

The heavy clouding formation of precipitate, thickening, and acid production took place in the serum from all but four of the cases of pneumonia. In two of these instances the blood was drawn after the crisis; and in the others on the day of the crisis and during a post-pneumonic empyæma respectively. Table I gives the days upon which the blood was drawn.

The reaction just described was not found to be specific for pneumonic sera. Practically the same alterations were observed when pneumococci were grown in the sera from two cases of general streptococcus infection, from a case of gonococcus endocarditis, and from a case of acute articular rheumatism. Slight clouding and moderate quantities of acid were formed with the sera from the five cases of chronic nephritis and uræmia. With serum from the last disease it required from 0.1 to 0.2 c.c.

$\frac{1}{10}$ N. sodium hydrate to neutralize 1 c.c. of serum. In the sera from three patients suffering with tuberculosis there was only a faint cloud and no acid. Two of these were cases of acute tuberculous pneumonia. It would be interesting to investigate this subject further. No acid, and very faint cloud were produced in beef and horse serum. Traces of acid appeared in calf serum, while in normal rabbit serum the reaction was definitely acid.

TABLE I.

ACID PRODUCTION BY GROWTH OF PNEUMOCOCCI IN PNEUMONIC SERUM.

Day of Disease on which Blood was Drawn.	No. of Cases.	Acid Production after 4–6 Days' Growth 36.5° C.
2d	1	+ + +
4th	3	+ + +
6th	3	+ + +
7th	2	+ + +
9th	1	+ + +
10th	1	+ + +
Day of crisis	1	+ −
4th day after crisis	1	+ −
5th " " "	1	−
Undetermined length of time, empyæma	1	+

Besides blood serum, pleural and spinal exudates were examined to determine if in these fluids acid was also formed. One cloudy pleural exudate from which the pneumococcus was recovered in cultures gave a moderate acid reaction both before and after growth at 36.5° C. One pleural exudate from which streptococci were recovered in cultures gave no acid reaction even after several days' growth at 36.5° C. The spinal fluid from two cases of meningitis caused by the pneumococcus gave no acid reaction. The spinal fluid from seven cases of meningitis due to the meningitis coccus gave no acid reaction, and the spinal fluid from one case of tuberculous meningitis (tubercle bacilli were demonstrated in the fluid) gave no acid reaction.

There is then some substance which makes its appearance in the blood serum under certain conditions and from which the pneumococcus is capable of forming large quantities of acid.

This substance is present with great constancy and in large amounts during an attack of pneumonia, but seems to disappear rapidly after the crisis. It may be present in general streptococcus and gonococcus infections, in acute articular rheumatism, and in comparatively small amounts in uræmia and chronic nephritis. So far as the present observations go, it has not been demonstrated in cases of pulmonary tuberculosis, in the serum from normal individuals, or in horse or beef serum. Traces could be shown to exist in calf and rabbit serum. Apparently the substance does not pass readily from the blood into exudates, for only small quantities of acid were formed in the pleural exudates caused by the pneumococcus, and none in the cerebro-spinal exudate. The nature of the substance could not be discovered. Almost certainly it is not glucose, for the fresh serum did not give the reaction for this sugar, and the large quantity of acid formed precludes the idea that it may have arisen from traces of glucose. It has been shown by Hiss [6] that the pneumococcus is capable of fermenting a long list of carbohydrates including the starches, such as inulin, which is not acted upon by streptococci. He suggests further that glucoproteids and nucleo-proteids may also be used in the complicated metabolic processes of the pneumococcus.

Rosenow noted that if pneumonic serum was heated to 56° C. and then used as a culture medium for the pneumococcus, the amount of acid formed was much less than if unheated serum was employed. The same results were obtained in a number of our cases. If, however, the serum was heated to 56°–60° C. for a greater length of time, usually one or two hours, the growth of the pneumococcus produced firm clotting of the serum within two to three days. This coagulum dissolved on the addition of alkalies. The same experiment was repeated several times. If by the growth of pneumococci in any blood serum, acid was produced in such amounts that it required from 0.2 c.c. to 0.6 c.c. of $\frac{1}{10}$ N. sodium hydrate to neutralize 1 c.c. of serum, heating to 57°–60° C. produced a firm white coagulum. This occurred whether the serum was heated before the growth of the pneu-

[6] *Jour. of Exper. Med.*, 1905, vi, 317.

mococcus or afterwards. Heating of the serum alone to 57° produced no visible change. Heating to 69°–72° C. gave rise to a firm jelly-like, clear clot. Without heating, the growth of pneumococci produced acid but gave rise to no coagulum. The coagulum was always soluble in an excess of alkali. When the serum was neutralized before heating, coagulation did not take place until the serum was heated to 65°–70° C.

Further experiments showed that the addition of definite amounts of lactic acid to several different sera gave rise to a coagulum when the serum was heated to 57° C., exactly like that obtained with the serum in which the pneumococcus had grown and produced acid. If, however, acid was added below a certain limit or above another limit, coagulation did not take place on heating. The amount of acid required was exactly that produced in the pneumonic sera by the growth of the pneumococcus, as the following table shows.

TABLE II.

Serum.	Amount of $\frac{1}{10}$ N. Sodium Hydrate Required to Neutralize 1 c.c. of Serum after Addition of Lactic Acid.	Temperature at which Serum Coagulates.
Beef	.15	72° C.
" "	.6	57° "
Chronic Nephritis 15	.1	72° "
" " "	.4	57° "
" " "	1.0	57° "
" " 2	.15	65° "
" " "	.4	57° "
" " "	.9	65° "
" " "	1.2	75° "
" "	.2	70° "
" "	.35	57° "
" "	.6	57° "

The fact that comparatively large quantities of acid could be added to, or produced in pure serum without the coagulation of the serum, suggested that the clotting of the serum-inulin water of Hiss was not entirely dependent upon the acid formed by the fermentation of the carbohydrate.

Beef serum was used in the preparation of the latter medium.

The blood was carefully collected and the serum kept under sterile conditions. Before making up the medium, specimens of the serum were incubated at 37° C. to make sure that the fluid was not contaminated. To this unheated serum sufficient quantities of sterilized inulin water were added to give the final mixture the proportions as described by Hiss. Pneumococci grown in this unheated serum-inulin water gave rise to large quantities of acid, but produced no coagulum. The medium remained absolutely fluid though a little turbid. For the coagulation of this medium, heating at least above 57° C. is absolutely essential.

That blood serum when diluted with two parts of water does not coagulate on boiling seems to be unexplained. But this heating must have some effect upon the serum. The fluid appears almost clear, and if passed through a Berkefeldt filter becomes absolutely limpid. Nevertheless, this serum differs from unheated serum in the fact that by the addition of small quantities of acids coagulation takes place immediately. Even pure albumen may be sterilized in a Koch autoclave without fear of coagulation, provided the albumen has been previously diluted with two to three parts of water. If, however, certain substances are added, coagulation takes place promptly. Attempts were made to grow pneumococci in pure diluted egg albumen. Mixtures of one part of egg albumen and two parts of water were sterilized at 100° C. The mixture remained absolutely fluid but it was found that pneumococci would not thrive in the medium. Salt and peptone were added to the albumen water, but it was impossible to sterilize this medium on account of the coagulation which took place.

It seems possible therefore that when serum diluted with water is heated to 100° C. the albumens and globulins are altered and are perhaps present in a so-called colloidal state. In this condition, additions of small amounts of acids cause rapid precipitation of the albumens and globulins and hence produce coagulation of the medium.